VOLUME 102 NUMBER 2

THE
BOTANICAL GAZETTE

December 1940

USES OF THE FEULGEN REACTION IN CYTOLOGY. II. NEW TECHNIQUES AND SPECIAL APPLICATIONS

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(WITH PLATES I–III)

Introduction

A previous paper (11) dealt with the effect of fixatives on the Feulgen nucleal stain, the causes of reported negative reactions, and the general applications of the stain. The present paper deals with various new methods made possible by the Feulgen reaction and the use and value of these methods in the study of chromosome form and structure.

The Feulgen reaction has made possible a new and useful method, the squash technique, whereby root tips, ovules, and anthers are fixed and stained *in toto*, and then spread out by squashing into a layer one cell thick. It eliminates the laborious and time-consuming imbedding and sectioning processes. This squash method was first employed by HEITZ (8) with root tips. He called it the *Nucleal-quetschmethode*. The squashing is made possible by the preliminary hydrolysis with HCl, necessary for the Feulgen reaction. This hydrolysis brings about dissolution of the pectic middle lamella and allows the cells to separate.

Squash techniques have numerous special applications and uses. The precise sharp stain, coupled with the spreading and flattening of the chromosomes, has made possible the study of prophase stages in certain plants where they have never been properly seen up to the present. Likewise the results in revealing the spiral structure of the chromosomes have been far superior to those obtained with other stains and methods. In this paper no attempt is made to work out the details of the structure of the chromosomes in any particular form. Rather, examples from various forms are chosen to indicate the wide range of application of the methods.

Methods

Anther squash

Since the publication of the anther squash technique (9), improvements have been made in the method. The following procedure has been found very satisfactory with material at all stages of meiosis. Anthers are dissected out, fixed with some suitable fixative, and washed. They are stained by means of the Feulgen reaction, bleached in SO_2 water, and transferred to a drop of 50 per cent acetic acid on a slide. A cover slip is put on and the anthers crushed by tapping and then applying pressure. The preparation is set aside for some time, 10–60 minutes, until it begins to dry at the edges. This period of drying is necessary to allow the loose cells to adhere to the slide and cover slip. Care must be taken not to let the preparations dry out completely, as the material will become desiccated and ruined wherever air comes in contact with it.

After the partial drying the cover slip is lifted off. This can be done with forceps if the cover is left protruding slightly over the edge of the slide. The cell debris is removed and both slide and cover slip dehydrated by flooding with dioxane, which is changed twice at 2-minute intervals. A drop of dioxane balsam is added and the cover slip replaced. If considerable material is present, a new cover slip is put on the slide and the original cover slip put on a new slide in order to ensure that the cells will not overlap.

The value of the anther squash technique lies mainly in the fact that it provides a means of spreading out the chromosomes so that each individual can be seen. This is demonstrated by the photomicrographs (figs. 1–8).

Anther smear squash

With the anther squash method the spiral structure of the chromonemata cannot be studied because pre-treatment before fixation is usually necessary to loosen the chromonematic coils so as to make their structure evident. The pre-treatment methods do not work successfully on whole anthers, so they must be smeared. In smear preparations the chromosomes are usually not spread out and separated sufficiently to allow the best observation. To overcome this the following method is employed. The anthers are smeared on a slide and pre-treated by one of the many methods for this purpose. The most satisfactory results have been obtained with sodium cyanide, 2^{-5} or 2^{-6} molecular solution, applied for 15–30 seconds (a modification of Oura's method, **18**). The preparations are then fixed, washed, stained, and bleached. A drop of 50 per cent acetic acid is added to the slide, a cover slip put on, and slight pressure applied. The rest of the procedure is the same as that described for the anther squash technique. The amount of tapping or pressure to be used must be determined by experience because it is easy to apply too much and flatten the chromosomes beyond recognition. Also

there is danger of the chromosomes becoming distorted or damaged if the cover slip is moved laterally owing to tapping with blows not delivered perpendicular to the surface.

Root tip squash

The method used by Heitz with root tips allows only temporary preparations to be made, as they are mounted in acetic acid. Methods of making the preparations permanent, such as those of Klingstedt (15) and Darlington and La Cour (4), are troublesome and complicated, and in my experience not satisfactory. The details of making permanent root tip squash preparations are given elsewhere (10). This method involves treatment with ammonium hydroxide before hydrolysis, to remove pectic compounds of the cell walls. This additional step is necessary to allow the cells to separate easily when the squashing is carried out in balsam after dehydration with dioxane. Such root tip squash preparations are valuable for making rapid somatic chromosome counts and for study of the details of the morphology of somatic chromosomes.

The root tip squash technique has frequently been criticized because preparations made by this method rarely show polar views of metaphase plates, thus making chromosome counts difficult. This is due to the fact that most of the cells are oriented with their spindles parallel to the long axis of the root. Polar views can sometimes be seen at the growing point, where the cells are not all oriented in the same direction. Frequently polar views can be obtained by moving the cover slip slightly and rolling the cells over. A more certain method is to cut thin freehand transverse sections of the root before fixation. These sections can either be treated by the method given for root tips (10) or they can be squashed in acetic acid, where they can be examined and counts made. If it is desired to make permanent preparations from the acetic acid mounts, the method described for the anther squash works very well. The previous method (10) is preferable for permanent mounts because there is no chance of disarranging or losing any of the cells, as the cover slip is never removed.

The squash method, using transverse sections of the root, is particularly valuable in making chromosome counts in polyploids and plants with large chromosome numbers. The chromosomes are well spread out and they all lie in one optical plane, which is rarely the case with paraffin sections. This is shown by the photograph (fig. 9) of a 60-chromosome *Gladiolus*. Burrell (2) has recently described a method similar to this but he uses acetocarmine as the stain.

In certain plants the root tips are so small that it is difficult to make freehand sections. With such plants, microtome sections 15–20 μ thick can be made from imbedded material and mounted on slides in the usual way. After treatment with the Feulgen reaction, they can be squashed in acetic acid and dehydrated in dioxane as before. This allows the chromosomes to be flattened and spread out,

which is impossible in an ordinary section. This method is needed only in extreme cases and usually the ordinary squash method or transverse freehand sections will provide all that is required.

Ovule squash

By means of this technique megagametophyte development can be traced from the gynospore mother cell up to fertilization and beyond, without the necessity of cutting sections. It is particularly valuable because the megagametophyte is kept as a unit and not distributed over a number of sections.

The following method has been developed. The ovules are dissected out, fixed, washed, and stained with the Feulgen stain. Vials are used to carry the material through all processes. It is essential to remove as much of the tissue around the ovules as possible. Vascular tissue in particular will carry air and water into the leuco basic fuchsin and oxidize it to basic fuchsin, producing a heavy stain in the cytoplasm which can hardly be removed. The same effect is produced after insufficient washing with a fixative containing formalin. The ovules are transferred from the SO_2 water to a drop of acetic acid on a slide and crushed under a cover slip. Permanent preparations are made in the same way as with the anther squash technique.

It has been possible with this method to trace completely megagametophyte development in *Lilium longiflorum* var. *formosum*. The nuclei and chromosomes stand out vividly in the colorless cytoplasm of the megagametophyte (fig. 10). Mature megagametophytes tend to break and collapse, owing to the large vacuoles present at this stage, but this does not happen in all cases.

How far the application of this technique can be carried is not known. It is limited mainly by the size of the ovules. In many of the angiosperms the ovules were too small to be handled. In *Pinus* the other extreme was found; too much cytoplasm was present, and the egg nucleus—which is not heavily stained owing to its large volume and dispersed chromatin—could not be clearly seen. This technique holds great promise and the limits of its use will be determined only by further investigation.

Summary of methods

In summing up the methods given, it may be said that they are all modifications of one general procedure. This is the basic procedure for the squash technique, and in many cases it must be altered or augmented by additional procedures to make it applicable to various types of materials. For the sake of conciseness, this general squash method is listed as follows:

1. Dissect out the structure desired, remove as much of the surrounding tissue as possible, and fix in some suitable fixative.
2. Wash thoroughly (vials are convenient carriers for the material and the fluids

can be changed with a pipette). If a fixative containing osmic acid is used, bleaching with hydrogen peroxide can be carried out at this stage.

3. Hydrolyze for 5 minutes in normal HCl at 60° C., using a water bath.
4. Stain in leuco basic fuchsin (fuchsin sulphurous acid) for from 30 minutes to overnight.
5. Bleach in three changes of SO_2 water of 10 minutes each.
6. Transfer a single piece of the material to a drop of 50 per cent acetic acid on a slide.
7. Squash the material under a cover slip by tapping with the end of a pencil and applying pressure with the thumb, using a piece of filter paper to protect the cover slip from finger prints.
8. Let the preparation stand 10–60 minutes until it begins to dry around the edges of the cover slip.
9. Remove the cover slip and dehydrate both slide and cover by flooding with dioxane, three changes of 2 minutes each.
10. Add a drop of Canada balsam and replace the cover slip.

Special applications

TO REVEAL SATELLITES.—The demonstration of satellites is a matter of proper fixation rather than of the technical methods employed. With certain fixatives they show up well in sectioned or smeared material, but frequently they are lying under another chromosome, or some similar condition obscures them. This difficulty can usually be overcome by means of the squash methods. With root tip squashes the chromosomes of the complement can be so well spread out that each individual is separate from the others. The same applies to pollen grain smears at the time of the first mitosis.

The smear squash method has proved very satisfactory with pollen grains, since they do not become contracted and shriveled as is frequently the case when balsam is added. This is probably due to the fact that a large area of the pollen grain wall is stuck to the glass, thus holding it there and allowing the balsam time to penetrate and fill the large vacuole before collapse of the wall can take place.

In mitotic prophase preparations stained with the Feulgen method the nucleolus is colorless, in contrast to the dense color it takes with other stains. This enables the satellites to stand out clearly against the colorless body of the nucleolus, showing their structural details. Yet it is often convenient to have some means of locating the satellites. This can be done by means of a counter stain of fast green, which stains the nucleolus a pale transparent green and does not interfere with the Feulgen stain of the chromosomes (fig. 11). The counter stain can be applied by placing the slide or material, following the SO_2 bleach and washing, in a 0.1 per cent solution of fast green for from 2 hours to overnight. They are then rapidly

washed, and the squashing in acetic acid and subsequent treatment carried out. SEMMENS and BHADURI (**19**) describe a technique for the differential staining of nucleoli and chromosomes by means of the Feulgen reaction and light green. Their method seems unnecessarily complicated, and in my experience the results obtained are no better than those with the simple aqueous solution. Fast green is used rather than light green because it is less likely to fade.

Normally with the Feulgen stain I use color filters to increase the contrast and also the definition. The combination of Ilford 3 and 5 or Wratten B and E filters (green and orange) has been found to give the best results. When these combinations are used with nucleoli stained with fast green, however, they are almost invisible because of low contrast. The orange filter alone gives a satisfactory contrast in chromosomes, satellites, and nucleoli. Other combinations also give excellent contrast (figs. 11, 12).

TO CLEAR UP MEIOTIC PROPHASE STAGES.—The anther squash method is particularly valuable for study of the prophase stages of meiosis. This method is indispensable in many plants, such as certain Liliaceae, where it is difficult to smear the anthers in early prophase since the cells tend to stick together in a tissue formation and will not adhere to the slide. Nuclei at the early prophase stages can be flattened and the chromonematic threads well spread out. They are not pulled out of place or distorted as is frequently the case with smears. The selective staining of the Feulgen reaction clearly reveals chromomeres of different sizes and shapes, with an unstained portion of the chromosome thread between them (fig. 13). KAUFMANN (**14**), KOSHY (**16**), and NAITHANI (**17**) attribute the beaded appearance of the chromosome to the intertwining of two threads, the chromomeres representing merely optical twists of the threads which have been exaggerated by the action of the fixatives. If such were the case the portion of the thread between the chromomeres would be stained. The Feulgen reaction demonstrates the presence of chromomeres and shows that they have a different chemical composition from that of the rest of the chromosome thread.

This technique also shows clearly the details of the chiasmata at diplotene. With especially favorable material, like the spermatocytes of grasshoppers, the crossing-over of the chromonemata is so clear that it appears almost diagrammatic (fig. 14). On the other hand, in some forms such as *Gasteria* it has been impossible up to the present to obtain a clear picture of chiasmata, owing to the fact that the chromosomes are labile and tend to run together, losing their individuality. In such forms the diplotene and diakinesis stages are usually referred to as the diffuse stage. With the Feulgen stain, used after Carnoy's fixative, which fixes the chromosomes before they get a chance to coalesce, it is possible to discern the chiasmata. A comparison of figure 15 with the drawings of TAYLOR (**20**) and TUAN (**22**) of a similar stage in *Gasteria* will make this clear. This method may prove useful in

forms such as *Tradescantia* and *Oenothera*, where preparations of the diplotene stage have been unsatisfactory. Preliminary work has been carried out on these forms with promising results.

TO STUDY CHROMOSOME STRUCTURE.—Preparations showing the spiral structure of the chromosomes can be produced by the pollen mother cell smear method and particularly the smear squash method. With the Feulgen stain the chromonema is revealed clear cut and sharp in a colorless matrix. This finding is opposed to most, if not all, previous reports. HUSKINS and SMITH (12) and GEITLER (6) report that the Feulgen nucleal stain gives no differentiation between the chromonema and its matrix. BAUER (1) and ZEIGER (24) report that both matrix and chromonema are nucleal positive. In no case in which material was fixed to reveal the spiral structure of the chromosomes was the matrix stained. It might be argued that in a chromosome which does not show a coiled chromonema, the matrix is also stained, since the whole chromosome appears homogeneously stained. The homogeneous appearance is almost certainly due to the tight coiling of the chromonema, which prevents the resolution of the different gyres. When the matrix is swollen as the result of pre-treatment, the coils are loosened and the gyres can be resolved; then the chromonema can be distinguished from the matrix. In cases where fixation has resulted in a vacuolated structure, this is of course revealed by the stain. Such a structure must be looked upon as an artifact.

With such Feulgen stained material the details of both major and minor coils at the first metaphase and anaphase are revealed (figs. 16, 17). The separation of the daughter chromonemata at the onset of the anaphase shows up sharply. Coiling in second meta- and anaphase chromosomes (that is, the minor coil), which has been revealed in comparatively few cases and then only by the aceto-carmine method of temporary staining, can be shown with the Feulgen stain (fig. 18).

The question of coiling in somatic chromosomes has led to marked divergence of opinion. The view that the chromonemata form two interlocking coils in the anaphase has been advanced by KAUFMANN (13), KOSHY (16), NAITHANI (17), TELEZYNSKI (21), TAYLOR (20), TUAN (22), and others. GEITLER (5, 7), UPCOTT (23), and DARLINGTON and LACOUR (4) have reported and illustrated a single coiled structure essentially similar to that found in meiosis. The aceto-carmine method was used by GEITLER and UPCOTT to reveal this structure, while DARLINGTON and LACOUR used the Feulgen method. Judging from the photographs of the latter, the results obtained have not been particularly favorable. In this laboratory the single coiled structure has been demonstrated through the use of the Feulgen method by COLEMAN (unpublished) in the pollen mitosis of *Gasteria trigona*, and by GOPAL-AYENGAR (unpublished) in the pollen tube mitosis of *Scilla* species.

Other stains will stain the chromonemata of chromosomes, but they are not so selective, and the preparations lack the precise staining so characteristic of the Feulgen stain and so necessary for a study of the details of chromosome structure. Moreover, by means of the Feulgen stain the chemical differentiation of the chromonema from its matrix has been definitely demonstrated.

WITH TRYPTIC DIGESTION.—Recent investigations by CASPERSSON (3) have shown that it is possible by means of tryptic digestion to remove the nucleoproteids from the chromosome, leaving only the thymonucleic acid. The digestion also removes any extra-chromosomal proteids in the nucleus as well as those of the cytoplasm. CASPERSSON found, by treating cells with lanthanum acetate, that the nucleic acid could be precipitated as an insoluble compound which he calls lanthanum nucleinate. The material is then subjected to digestion by the enzyme trypsin, which removes the protein of the matrix but leaves the thymonucleic acid intact.

CASPERSSON studied mainly the salivary gland chromosomes of *Drosophila* and the spermatocytes of certain grasshoppers. He did this by means of photomicrographs in ultra-violet light with wave lengths of 2560–2750 A. The differentially greater absorption of light rays of this wave length by nucleic acid led to photomicrographs showing great detail in the bands of salivary gland chromosomes, to which areas the thymonucleic acid is restricted. Previous digestion gave preparations which under the ultra-violet microscope resulted in great detail and clearness. CASPERSSON did not use his technique in a study of chromosome structure in plant cells.

As ultra-violet microscope equipment is expensive and therefore not generally available, it was felt that the possibility of combining CASPERSSON'S digestion method with the Feulgen technique should be investigated. It had first to be ascertained whether the precipitation of thymonucleic acid as a lanthanum salt interferes with the action of hydrolysis. Experiments showed that this is not the case and that the Feulgen reaction can be carried out successfully on material treated by CASPERSSON'S digestion method.

A modification of CASPERSSON'S method was introduced. Instead of using a mixture of fresh trypsin from the mucous lining of pig's gut and enterokinase from pig pancreas, a dried commercial preparation of trypsin was found to be equally satisfactory. Smears of pollen mother cells of *Tradescantia virginiana* were pretreated and fixed as before. The slides were then left overnight in a 0.1 per cent solution of lanthanum acetate. They were next placed in a 1 per cent solution of trypsin which contained a trace of lanthanum acetate, to prevent dissolution of the thymonucleic acid. They remained in this solution for 24 hours at 37° C. The trace of lanthanum acetate necessary for the reaction was provided by not washing the slides when transferring from the lanthanum acetate solution to the trypsin

solution. When five slides were placed in a Coplin jar at one time, sufficient lanthanum acetate was carried along to keep the nucleic acid in an insoluble form.

This combination of time of digestion and concentration of trypsin was sufficient to remove most of the matrix of the chromosomes and some of the cytoplasm. By increasing the time or concentration more complete digestion could be brought about, but if it was carried too far there was nothing left to hold the chromonemata to the slide. Preparations treated in this way were a considerable improvement over non-digested preparations. The clear but light refracting cytoplasm of the background is removed and the halo effect of the matrix is gone, leaving the chromonemata standing out naked and sharp. There is nothing to obscure the most minute details of the structure and coiling of the chromonemata; thus this type of preparation is unequaled for investigation of chromosome structure. For ordinary work such a highly refined smear technique is not necessary, but for future investigation of chromosome structure this method should prove valuable.

Summary

1. Procedures are described for use with the squash technique, whereby anthers, ovules, and root tips are fixed and stained *in toto*, then spread out into a layer one cell thick by squashing.

2. The smear squash method of studying chromosome structure, particularly the minor coil, is described. Contrary to most previous reports, the Feulgen stain was found to stain only the chromonema and not the matrix of the chromosome.

3. The Feulgen stain can be used with marked success after the trypsin-lanthanum digestive method of CASPERSSON, which removes the matrix of the chromosomes as well as other cell proteids. Preparations made in this way are particularly sharp and clear, and this method holds promise for the investigation of chromosome structure.

4. The anther squash technique is the first to reveal at all clearly the chiasmata and individuality of the chromosomes in forms that have a diffuse stage at diplotene and diakinesis.

5. Satellites can be revealed with remarkable clarity by the root tip squash method, and the anther smear squash method when used with pollen grains. A counter stain of fast green is used to differentiate the nucleolus.

The writer wishes to express thanks to Dr. L. C. COLEMAN for assistance and the use of many of his preparations and to Dr. D. H. HAMLY for help with photography.

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EXPLANATION OF PLATES I–III

PLATE I

FIGS. 1–6.—Anther squash preparations of *Lilium longiflorum* var. *formosum* fixed in Carnoy's fluid and hydrolyzed 5 minutes. All ×1000.

FIG. 1.—Pachytene stage.

FIG. 2.—Early diplotene showing crossing-over of chromonemata.

FIG. 3.—Diakinesis; chromosomes well spread out.

FIG. 4.—Meta-anaphase.

FIG. 5.—Anaphase I showing chromosomes well spread out.

FIG. 6.—Interphase showing opening out of major and minor coils.

PLATE II

FIG. 7.—Metaphase II of *L. longiflorum* var. *formosum* showing complete chromosome complement with chromosomes (some terminal and others median) attached at primary constriction. ×1000.

FIG. 8.—Anaphase II of same, with chromosomes well spread out. ×1000.

FIG. 9.—Root tip squash preparation of 60-chromosome *Gladiolus*, fixed in Carnoy. (From preparation made by G. S. SWAIN.) ×1500.

FIG. 10.—Ovule squash preparation of *Lilium longiflorum* var. *formosum* showing megagametophyte with 12- and 36-chromosome nuclei at metaphase. Belling's fixative. ×500.

FIG. 11.—Pollen grain smear of *Aloe vera* showing satellites on nucleolus. To bring out sufficient contrast between the chromosomes and nucleolus, which is stained with fast green, the following color filters were used: Ilford 5, Wratten E, and Chant's signal green glass. Fleming-Heitz fixative. ×1400.

FIG. 12.—Same nucleus as in fig. 11 taken with ordinary color filter combination used with Feulgen stain (Ilford 3 and 5). Nucleolus scarcely visible.

FIG. 13.—Anther squash preparation of *Veltheimia viridifolia* showing zygotene stage. Chromomeres appear as beads with unstained portion of thread between them. Fixed in Carnoy. ×1500.

PLATE III

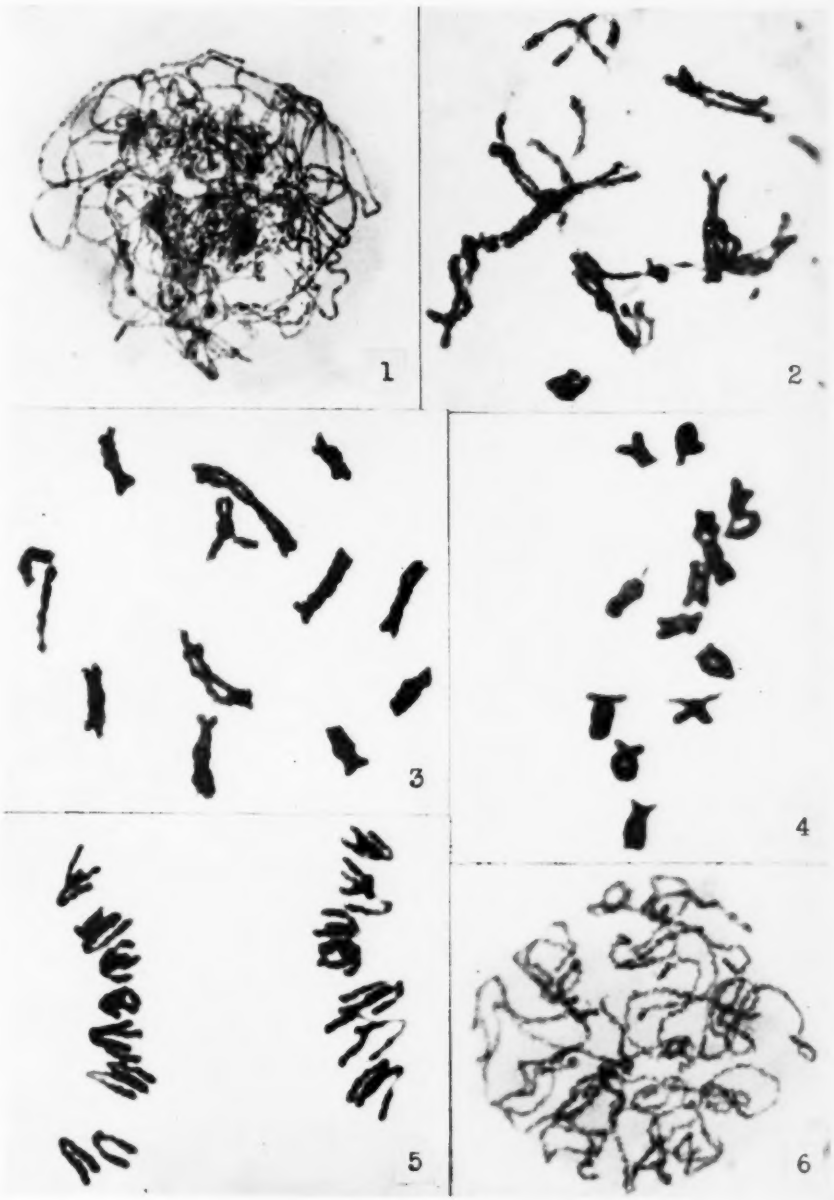
FIG. 14.—Serial photographs of different focal levels of diplotene stage of the grasshopper, *Melanoplus femur rubrum*, showing well defined chiasmata. Preparation made by squashing the testes tubules in the same way as anthers. ×1000.

FIG. 15.—Anther smear squash of the rarely seen diplotene stage of *Gasteria trigona*, showing chiasmata and chromosomes well spread out. Fixed in Carnoy. ×1000.

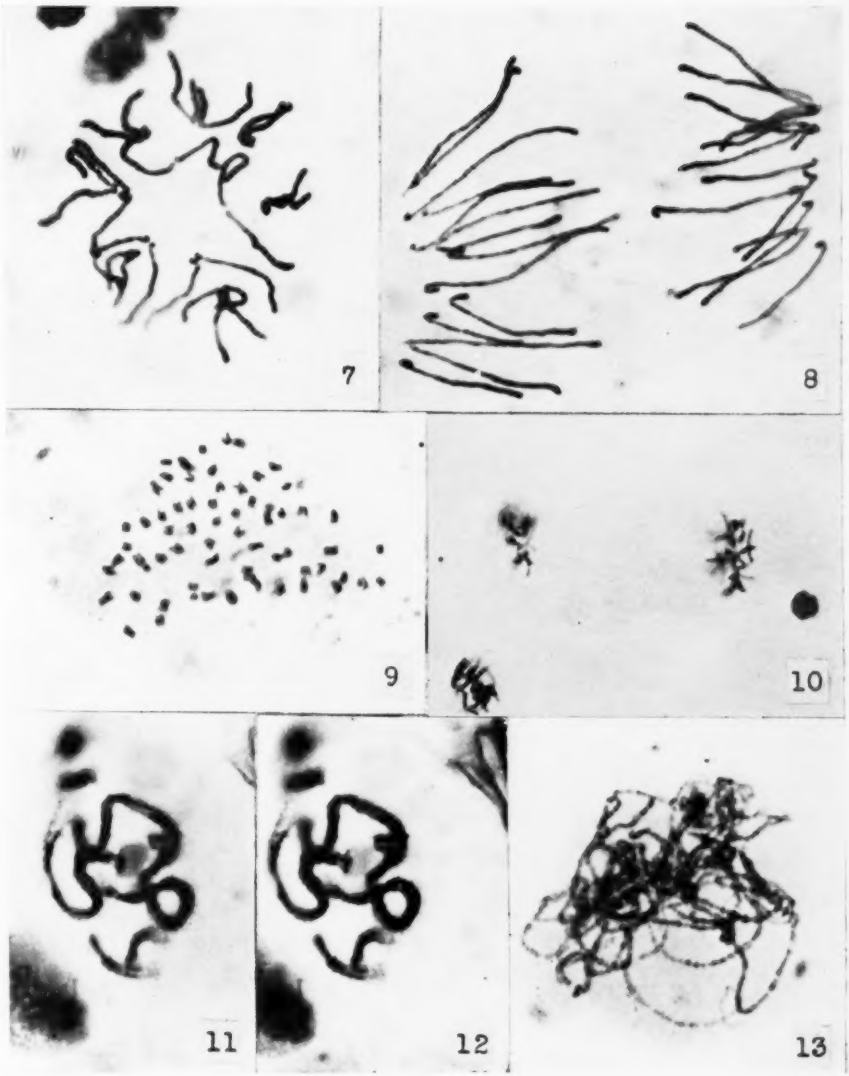
FIG. 16.—Anther smear squash of *Tradescantia virginiana* at metaphase I, showing minor coils of double chromonemata and connections between chromosomes in ring of four. Pre-treated 30 seconds with NaCN 2^{-5} molecular solution and fixed in Carnoy. ×1600.

FIG. 17.—Anther smear squash of early anaphase I of *T. paludosa* with minor coil revealed at points indicated by arrows. ×1300.

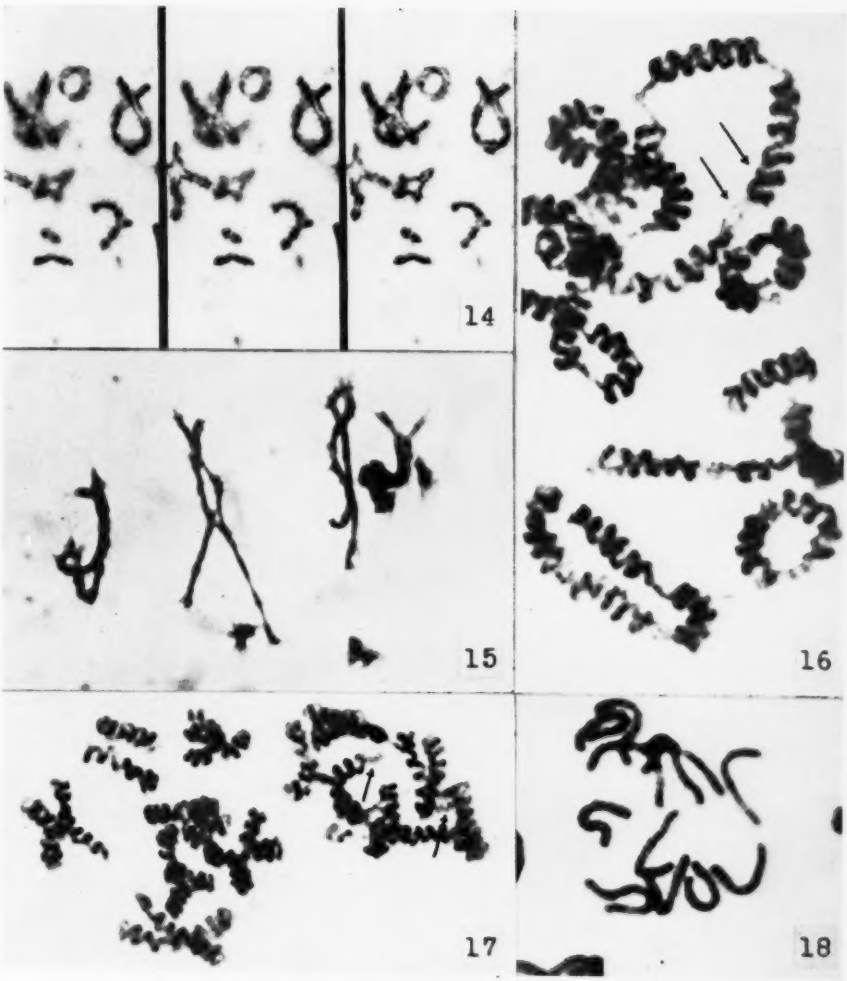
FIG. 18.—Anther smear squash of anaphase II of same, showing minor coil. Pre-treated in NaCN 2^{-5} molecular solution for 15 seconds and fixed in Navashin. ×1500.



HILLARY on FEULGEN REACTION



HILLARY on FEULGEN REACTION



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STUDIES OF CHROMOSOMAL ASSOCIATION AND BEHAVIOR AND OCCURRENCE OF ANEUPLOIDY IN AUTOTETRAPLOID GRASS SPECIES, ORCHARD GRASS, TALL OAT GRASS, AND CRESTED WHEATGRASS[1]

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(WITH THREE FIGURES)

Introduction

Although most polyploid species behave cytologically as allopolyploids, some appear to be autopolyploids. In an extensive review of the literature on this subject, MÜNTZING (8) emphasized the importance of autopolyploidy in evolution.

Among the many polyploid species of the Gramineae, at least four have been reported to show meiotic chromosome behavior characteristic of autopolyploids. The presence of quadrivalents at metaphase I has been reported by MÜNTZING (7, 9) in orchard grass (*Dactylis glomerata*), by VON BERG (1) in *Hordeum bulbosum*, and by KATTERMANN (3) in tall oat grass (*Arrhenatherum elatius*) and sweet vernal grass (*Anthoxanthum odoratum*). PETO (12) found both diploid and tetraploid races in crested wheatgrass (*Agropyron cristatum*), and the senior writer (unpublished) observed the regular occurrence of quadrivalents at meiosis in the tetraploid race of this species.

Cytological studies of naturally occurring autopolyploids are of both theoretical and practical importance. A comparison of the meiotic behavior of different species and of plants within these species should throw light on the effects of natural selection on chromosomal behavior. Such information will be particularly valuable for comparisons with induced autopolyploids. From a practical standpoint, a varietal improvement program can be outlined more intelligently when the cytogenetical behavior of the species is understood.

Material and methods

Microsporocytes were collected from six plants of crested wheatgrass, ten plants of tall oat grass, and three plants of orchard grass. The sporocytes were

[1] The quadrivalent frequency and percentage of metaphase I sporocytes showing univalents were determined for crested wheatgrass plants GH-1 and GH-2 and tall oat grass plants 162–1, 162–3, 162–4, 162–6, 162–10, and 162–11, while the senior writer was a member of the staff of the Division of Agronomy and Plant Genetics, University of Minnesota. All other phases of the investigation were conducted at the U.S. Regional Pasture Research Laboratory, State College, Pennsylvania.

[2] Associate Geneticist; [3] Agent.

fixed in acetic-alcohol (three parts absolute alcohol to one part glacial acetic acid) and left in this solution until examined. Aceto-carmine smear slides were used, and all data were recorded and photomicrographs made from the fresh slides. Best preparations were obtained from material killed the previous day, but fairly satisfactory slides were made from material that had been in the fixing solution more than a month.

The chromosome numbers of 116 plants of orchard grass were determined from paraffin sections of root tips, prepared as described previously for *Lolium perenne* (10). In determining the number of chromosomes, three or more of the best plates from each plant were sketched and the counts made from these sketches. In case of discrepancy the plates were reexamined or new ones sketched until the count seemed correct. In the tall oat grass and crested wheatgrass plants which were studied, chromosome numbers were determined from the microsporocytes.

Experimental results

Chromosome numbers

Of the 116 plants of orchard grass studied, 59 per cent had the euploid chromosome number of 28. The remaining 41 per cent were aneuploid, 22 per cent having 27, 12 per cent 29, and 7 per cent 30 chromosomes. Of the ten plants of tall oat grass studied, nine were euploid ($2n = 28$) and one had 27 chromosomes. Five of the crested wheatgrass plants had 28 chromosomes while one showed clearly more than 28. In this plant the chromosome number at diakinesis could be determined unquestionably in only two sporocytes, both of which showed 31 chromosomes.

Meiotic behavior

Mid-prophase.—The chromosomes at mid-prophase were found to be regularly associated in pairs but frequent changes of partners were observed, indicating some quadrivalent associations. Although it was not possible to analyze a complete nucleus at this stage, in some cases a single pair of homologues could be seen associated throughout their entire length, a type of pairing which should result in a bivalent at diakinesis and metaphase I. Quantitative data on the frequency of such pairing could not be obtained, but it is doubtful whether it occurs frequently enough to account for all the bivalents produced. Bivalents probably arise also as a result of failure of chiasma formation in certain paired segments of quadrivalent associations in prophase.

Diakinesis and metaphase I.—The frequency of quadrivalents and bivalents was determined from diakinesis, metaphase I, or both. When both stages were used in a particular plant, the results were similar. The data were therefore combined. Only nuclei which could be analyzed completely were recorded. The num-

ber of sporocytes in each plant showing various combinations of bivalents and quadrivalents, the average number of quadrivalents per plant, and the total and average for each species are given in table 1. All possible combinations of bivalents and quadrivalents, except 14 bivalents, were observed. In addition to

TABLE 1

NUMBER OF MICROSPOROCYTES SHOWING VARIOUS NUMBERS OF BIVALENTS AND QUADRIVALENTS

PLANT NO.	$^{12}II+^{1}IV$	$^{10}II+^{2}IV$	$^{8}II+^{3}IV$	$^{6}II+^{4}IV$	$^{4}II+^{5}IV$	$^{2}II+^{6}IV$	^{7}IV	TOTAL NO. OF CELLS	AVERAGE NO. OF IV
	ORCHARD GRASS								
11(13)	1	5	4	11	6	2	0	29	3.8
22(2)	3	2	2	11	6	4	2	30	4.2
138(3)	1	1	4	4	1	0	0	11	3.3
Total	5	8	10	26	13	6	2	70	3.9
	CRESTED WHEATGRASS								
GH1	1	4	6	6	4	1	1	23	3.7
GH2	2	6	5	5	6	1	0	25	3.4
4(1)	0	0	0	1	0	0	0	1	.....
4(4)	0	1	1	0	0	0	0	2	.....
9(1)	1	1	3	5	5	3	0	18	4.2
Total	4	12	15	17	15	5	1	69	3.7
	TALL OAT GRASS								
162-1	1	1	7	12	5	1	0	27	3.8
162-3	0	2	5	4	2	0	0	13	3.5
162-4	0	3	4	5	5	0	0	17	3.7
162-6	0	4	12	10	3	0	0	29	3.4
162-11	1	4	8	9	8	2	3	35	4.1
9(7)	1	0	0	3	3	1	0	8	4.2
12(3)	0	2	1	3	4	2	1	13	4.5
12(1)	0	0	1	1	1	1	0	4	4.5
4(4)	1	3	10	6	8	1	1	30	3.8
Total	4	19	48	53	39	8	5	176	3.8

the 315 sporocytes recorded in table 1, hundreds of nuclei at diakinesis and metaphase I were examined in which complete analysis was not possible. Since all these nuclei had one or more quadrivalents, sporocytes with 14 bivalents must occur rarely if at all. Sporocytes in diakinesis in orchard grass are shown in figure 1*A*, *B*. The quadrivalents and bivalents are clearly visible. The occurrence of 7

quadrivalents in some nuclei of each species indicates at least partial homology within each of the 7 sets of 4 in each species. In tall oat grass 162–11, of 76 nuclei examined at diakinesis one had a ring of 12 chromosomes plus 4 quadrivalents. This was the only sporocyte in any plant in which an association of more than 4 chromosomes was observed, indicating that the ring of 12 was the result of an aberration which occurred in a premeiotic division or in prophase of meiosis. The production of such a ring in this material would require two interchanges with one set of homologues in common.

Although the mean numbers of quadrivalents for the three species were very nearly alike, variation between plants within each species was found. The range of means for plants of orchard grass was 3.3 to 4.2, for crested wheatgrass 3.4 to 4.2, and for tall oat grass 3.4 to 4.5. Since determinations for a plant were made

TABLE 2

PERCENTAGE RING AND CHAIN QUADRIVALENTS SHOWING ADJACENT AND ALTERNATE TYPES OF ORIENTATION AT METAPHASE I

Type of orientation	Tall oat grass (plant no.)					Crested wheatgrass (plant no.)		
	162-1	162-4	162-6	162-11	Total	G.H. 1	G.H. 2	Total
Alternate	71.4	70.0	61.8	90.7	74.4	79.1	90.6	84.7
Adjacent	28.6	30.0	38.2	9.3	25.6	20.9	9.4	15.3
Number of quadrivalents	77	66	68	86	297	91	85	176

usually from a single collection of sporocytes and since the sporocytes were collected at different times, no valid estimate of error is available. It is impossible, therefore, to evaluate the significance of these differences between plants. Nevertheless they are suggestive of a possible trend in natural selection for different quadrivalent frequencies.

The majority of the quadrivalents observed at diakinesis and metaphase I were simple rings or chains with completely or nearly completely terminalized chiasmata. The type of orientation of these rings and chains in four plants of tall oat grass and two plants of crested wheatgrass was classified as to whether alternate or adjacent chromosomes were directed toward the same pole (table 2). Figure 1*C* shows a quadrivalent of the adjacent type and figure 1*D* shows quadrivalents the members of which are oriented in an alternate manner. The four plants of tall oat grass ranged from 61.8 to 90.7 per cent of the quadrivalents oriented alternately, while in the two plants of crested wheatgrass, 79.1 and 90.6 per cent of the quadrivalents were classed as alternate. The magnitude of the differences suggests that they may be significant.

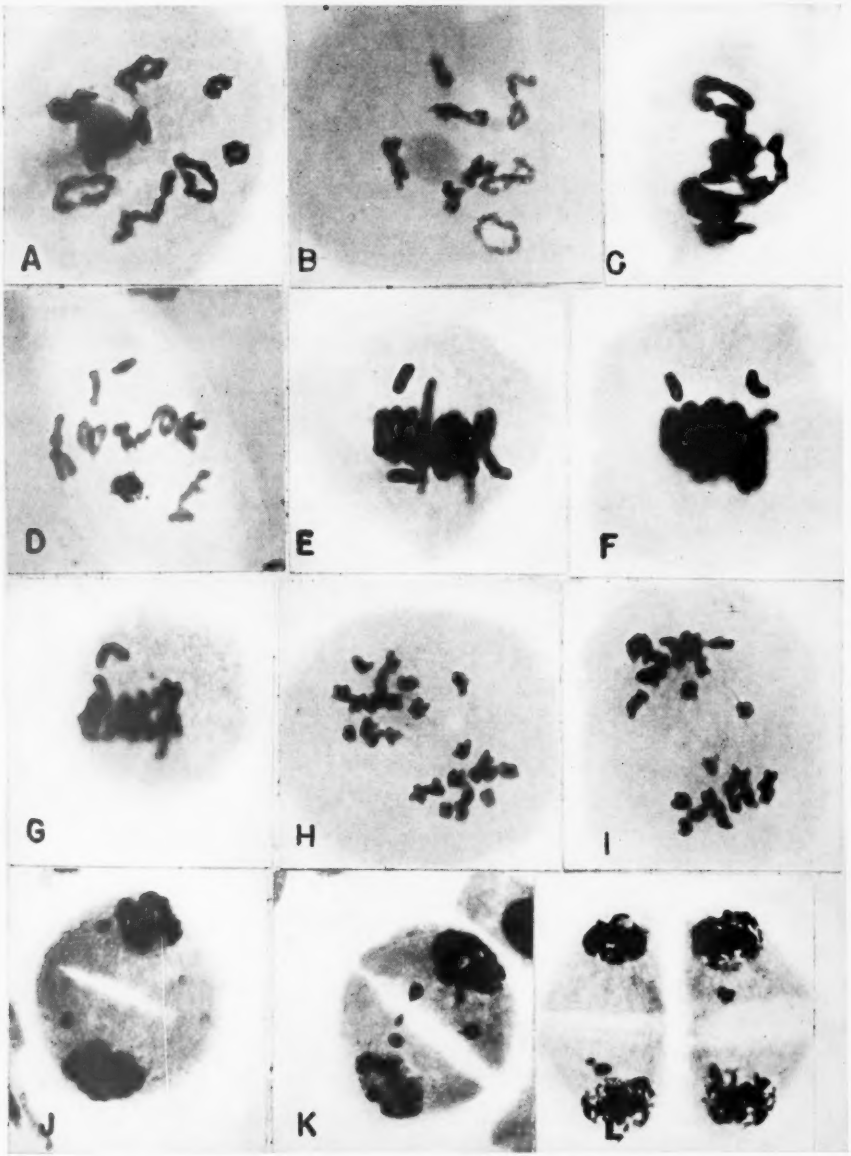

FIG. 1.—*A*, *B*, quadrivalents and bivalents at diakinesis; *C*, *D*, types of quadrivalents at metaphase I; *E*, *F*, *G*, univalents at metaphase I; *H*, *I*, lagging and dividing univalents at anaphase I; *J*, *K*, micronuclei at interphase I; *L*, quartet showing planes of meiotic divisions.

In addition to the sporocytes having only bivalents and quadrivalents, some were observed with one or more univalents at metaphase I. In obtaining quantitative data on percentage of metaphase I sporocytes with different numbers of univalents (table 3), an attempt was made to record only univalents which apparently had not been associated with other chromosomes by chiasmata. Some errors undoubtedly were made in distinguishing these univalents from those resulting from precocious disjunction. Also, univalents probably were obscured by the main group of chromosomes in some nuclei, so that the percentages given in table 3 may represent minimum values for that particular group of sporocytes. Large differences in the univalent frequency in different plants were found. Tall oat grass 12(3) was the only plant in the three species in which univalents were not observed, although the percentage of sporocytes with univalents was low in plants 162–3 and 162–11. The greatest frequency of single univalents was found in crested wheatgrass 4(6), but the percentage of nuclei showing one or more univalents was higher in 4(1). The frequency of metaphase I plates showing two univalents is considerably higher than would be expected owing to chance—on the basis of the frequency of nuclei with single univalents. This may have resulted from conditions within a given nucleus tending to decrease chiasma formation. Thus such a nucleus would frequently have two or more univalents which would not necessarily be homologous. In sporocytes with two univalents, however, the univalents were usually similar morphologically (fig. 1*E*, *F*), whereas those seen in different nuclei were frequently strikingly different in size and arm length (fig. 1*G*). Attempts to identify the chromosomes at this stage are admittedly unsafe. Nevertheless these observations suggest that the two univalents in a particular nucleus were frequently homologous. This indicates that when pairing in a homologous set at prophase is such that one chromosome fails to form a chiasma, there is a tendency for another chromosome of the same set to be excluded from chiasma formation, with the resulting formation of two univalents and one bivalent instead of one univalent and one trivalent.

ANAPHASE I.—In all plants in which anaphase I figures were examined, some showed one or more lagging univalents (fig. 1*H*, *I*). All univalents which seemed to be lagging sufficiently to prevent their inclusion in one of the daughter nuclei were on the equatorial plate or close to it and were clearly split longitudinally. In many cases the half chromosomes had already disjoined and were seen lying close to the two daughter chromosome groups. As may be seen in table 3, there is a tendency for the frequency of lagging univalents at anaphase I to be higher in those plants with a high frequency of univalents at metaphase I, indicating that unpaired chromosomes are unable to move in a normal manner to the pole, but lag behind, split equationally, and move to the poles as half chromosomes. It is impossible to estimate from these data what proportion of

the metaphase I univalents behave in this manner. Tall oat grass 12(3), which did not have univalents in any of the 184 metaphase I figures counted, had one lagging univalent in 5.2 per cent of the anaphase I nuclei examined. All plants except tall oat grass 12(1) showed a corresponding increase in frequency of ana-

TABLE 3

PERCENTAGE OF METAPHASE I SHOWING DIFFERENT NUMBERS OF UNIVALENTS AND PERCENTAGE OF ANAPHASE I SHOWING DIFFERENT NUMBERS OF LAGGING UNIVALENTS

Plant no.	Metaphase I							Anaphase I							
	No. examined	No. of univalents						No. examined	No. of lagging univalents						
		1	2	3	4	6	Total		1	2	3	4	6	8	Total
	Orchard grass														
11(13)	102	6.9	3.0	....	....	....	9.9	55	9.1	9.1	....	....	....	....	18.2
22(2)	172	5.8	0.6	....	....	....	6.4	48	14.6	8.3	2.1	....	....	....	25.0
138(3)	119	16.8	9.2	2.5	0.8	1.7	31.0	16	18.8	12.5	6.2	6.2	6.2	6.2	56.1
	Crested wheatgrass														
GH 1	500	7.0	0.4	....	....	....	7.4	.....	.....	.....	.....	.....	.....	.....	.....
GH 2	356	2.5	0.6	....	....	....	3.1	.....	.....	.....	.....	.....	.....	.....	.....
4(1)	103	16.5	16.5	2.0	4.0	....	39.0	84	30.9	3.6	1.2	.....	.....	.....	35.7
4(4)	121	8.2	10.7	3.3	0.8	....	23.0	60	16.7	3.3	.....	.....	.....	.....	20.0
4(6)	142	22.5	4.2	5.6	....	....	32.3	11	36.4	9.1	.....	.....	.....	.....	45.5
9(1)	135	18.5	11.1	....	....	....	29.6	3	66.0	.....	.....	.....	.....	.....	66.0
	Tall oat grass														
162-3	151	0.7	.....	....	....	....	0.7	.....	.....	.....	.....	.....	.....	.....	.....
162-10	661	7.3	2.0	....	....	....	9.3	.....	.....	.....	.....	.....	.....	.....	.....
162-11	600	0.8	0.5	....	....	....	1.3	.....	.....	.....	.....	.....	.....	.....	.....
4(4)	168	3.0	3.0	....	....	....	6.0	88	8.0	9.1	1.1	.....	.....	.....	18.2
9(7)	111	4.5	1.9	....	....	....	6.4	60	11.7	6.7	3.3	.....	.....	.....	21.7
12(1)	151	10.6	2.0	....	....	....	12.6	168	3.0	.....	.....	.....	.....	.....	3.0
12(3)	184	.....	.....	....	....	....	0.0	135	5.2	.....	.....	.....	.....	.....	5.2

phase I with single laggards as compared with the percentage of single univalents at metaphase I. When the total percentage of anaphase I nuclei showing one or more lagging univalents is compared with the total percentage of metaphase I with one or more univalents, a similar increase is noted in all plants except tall oat grass 12(1) and crested wheatgrass 4(1) and 4(4). In the latter two plants the differences between anaphase I and metaphase I were slight and may not be

significant. It seems probable that in some of the plants studied, lagging univalents arose from some source other than unpaired chromosomes at metaphase I. It is possible that in quadrivalents, particularly those the members of which are oriented in an adjacent manner, the disjoining chromosomes lie so far apart that forces of repulsion will be insufficient to move all chromosomes to the poles. Such chromosomes would lag and perhaps undergo longitudinal division. Other factors besides failure of chromosomes to pair and poor disjunction of quadri-

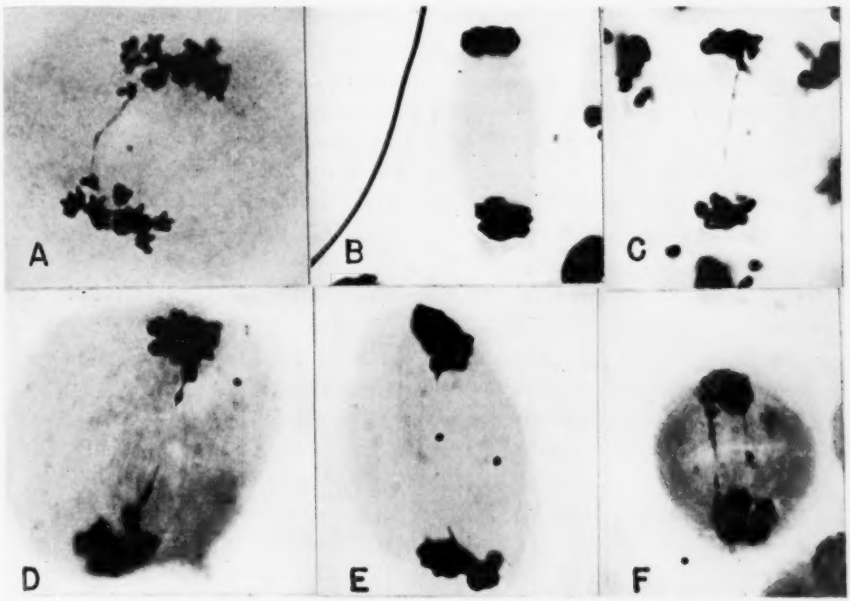

FIG. 2.—*A*, chromatid bridge and fragment at anaphase I; *B*, fragment at telophase I; *C*, *D*, *E*, chromatid bridges and fragments at telophase I; *F*, double chromatid bridge and single chromatid bridge at interphase I.

valents may be involved in the origin of lagging chromosomes. In one diploid plant of *Lolium perenne* L. the senior writer (unpublished) found a frequency of lagging univalents at anaphase I much too great to be explained on the basis of unpaired chromosomes at metaphase I. Quadrivalents were not found in this plant.

One of the striking features of anaphase I in the plants used in this investigation was the occurrence of chromatin bridges and acentric fragments (fig. 2*A*). These configurations appeared to be identical with those described by McCLINTOCK (**4**, **5**, **6**) and others as arising from crossing-over within an inversion. It

may be seen from table 4 that these configurations were observed in all but two plants, and in these plants only three and sixteen anaphase figures, respectively, were examined. In these same two plants telophase I nuclei clearly showed bridges and fragments. Crested wheatgrass 4(4) had some anaphase I figures with two bridges and two acentric fragments, indicating that they may have been heterozygous for two inversions. In tall oat grass 12(1) the high percentage of sporocytes

TABLE 4

PERCENTAGE OF ANAPHASE I SPOROCYTES SHOWING ONE AND TWO DICENTRIC BRIDGES AND ACENTRIC FRAGMENTS, AND PERCENTAGE SHOWING ONE ACENTRIC FRAGMENT

PLANT NO.	NO. EXAMINED	BRIDGE+FRAGMENT		FRAGMENT
		1	2	1
	ORCHARD GRASS			
11(13)	55	3.6	.....	.....
22(2)	48	10.4	.....	.....
138(3)	16	.....	.....	.....
	CRESTED WHEATGRASS			
4(1)	84	14.3	.....	.....
4(4)	60	16.7	1.7	3.3
4(6)	11	27.3	18.2	.....
9(7)	3	.....	.....	.....
	TALL OAT GRASS			
4(4)	88	4.5	.....	2.3
9(7)	60	1.7	.....	.....
12(1)	168	41.7	.....	10.1
12(3)	135	2.2	.....	.....

showing bridges and fragments suggests that it also may be heterozygous for more than one inversion, or that a long inversion permitting frequent crossing-over is involved. The assumption of heterozygosity for more than one inversion is supported by the occurrence of two bridges plus two fragments in one nucleus at telophase I.

INTERPHASE I.—The fate of the lagging and dividing univalents during the first division of meiosis can be determined approximately from the frequency of micronuclei found in the sporocytes at interphase I. Observations of late ana-

phase I and early telophase indicated that some of the half chromosomes resulting from the equational division of univalents were being included in the daughter nuclei. Others apparently lagging too far behind to be included would be left in the cytoplasm to form chromatin clumps or true micronuclei. Each lagging univalent at anaphase I has the potentiality of producing two micronuclei at interphase (fig. 1*J*). If all laggards were left in the cytoplasm, a given plant should have approximately twice as many micronuclei at interphase as lagging univalents at anaphase I. A comparison of the data given in table 5 with those given for anaphase I of the same plants in table 3 shows that this was not the case. The majority of the half chromosomes evidently were included in the daughter nuclei, as suggested from the observations just noted. Whether laggards formed micronuclei or were included in telophase I nuclei appeared to be conditioned simply by their chance position when the nuclear membranes were formed, and this must have operated similarly in different plants, since there was close relation between frequency of micronuclei and frequency of lagging univalents for the different plants. This relation is further evidence of the significance of the differences between plants in the meiotic irregularities studied. The chief discrepancy in this general relation is found in the four plants of crested wheatgrass. Since only three anaphase I figures were examined in plant 9(1), the discrepancy here has little significance. In plant 4(4) the percentage of sporocytes showing micronuclei was much lower in relation to the frequency of lagging univalents than in 4(1) and 4(6). The data on interphase I in the former plant were taken from a slide from a single anther in which approximately 50 per cent of the sporocytes were in metaphase I. The possibility exists that the normal sporocytes in any sac undergo division more rapidly than the abnormal and that those examined from plant 4(4) were selected from the more precocious group. In addition to the sporocytes recorded in table 5 for crested wheatgrass 4(6), one very abnormal telophase I was observed. Two groups of chromosomes had formed on one side of the division wall and one group on the other. In addition there were observed six chromatin clumps, each of which appeared to have arisen from a single half chromosome, two fragments, and one chromatin bridge.

A part of the sporocytes in each plant showed fragments and remnants of broken chromatin bridges at telophase I and interphase. Figure 2*B* shows a fragment at telophase I, while fragments and broken bridges are shown in figure 2*C*, *D*, and *E*. In general the relative frequency for different plants compared favorably with the data from anaphase I reported in table 4. In all plants, however, the percentage of sporocytes showing these configurations was lower at telophase I and interphase than at anaphase, probably because of observational difficulties. Apparently the presence of the fragment at these stages was frequently obscured by its inclusion in one of the daughter nuclei. The condition of

the bridge remnants was seen to vary, from cases in which the bridge had apparently just broken (fig. 2*C*, *D*) to cases where only slight protrusions from the interphase nuclei indicated that a bridge had existed previously (fig. 2 *E*). No doubt some of the latter cases were overlooked. McCLINTOCK (5) reported that in maize the majority of the chromatin bridges break, the broken ends of the sister half chromatids rejoining to produce bridges in anaphase of the first micro-

TABLE 5

PERCENTAGE OF SPOROCYTES AT INTERPHASE I SHOWING VARIOUS NUMBERS OF MICRONUCLEI

PLANT NO.	NO. COUNTED	NO. OF MICRONUCLEI IN SISTER CELLS OF SPOROCYTES							NO. OF MICRONUCLEI PER 100 SPOROCYTES
		0-1	1-1	0-2	1-2	2-2	2-3	2-4	
	ORCHARD GRASS								
11(13)	169	8.9	1.2	.....	.....	0.6	.....	.....	13.7
22(2)	78	11.5	5.1	1.3	2.6	.....	.....	1.3	39.9
138(3)	186	13.4	5.9	2.7	1.6	.....	1.1	0.5	43.9
	CRESTED WHEATGRASS								
4(1)	153	17.6	3.9	2.0	.....	.....	.....	.....	29.4
4(4)	168	1.8	0.6	.....	.....	.....	.....	.....	3.0
4(6)	184	20.6	7.6	1.1	1.1	0.5	.....	.....	43.3
9(1)	109	4.6	0.9	.....	.....	.....	.....	.....	6.4
	TALL OAT GRASS								
4(4)	126	4.0	2.4	.....	0.8	0.8	.....	.....	14.4
9(7)	225	9.8	4.4	.....	0.4	.....	.....	.....	19.8
12(1)	178	.....	1.1	.....	.....	.....	.....	.....	2.2
12(3)	232	1.7	0.4	.....	.....	.....	.....	.....	2.5

spore mitosis. On the other hand, EMSWELLER and JONES (2) found in *Allium* that the chromatin bridges did not break but disintegrated in the cytoplasm. The bridges observed in this material appeared to break in the same manner as described by McCLINTOCK (5). Studies of mitosis in the microspores in these plants have not been made. In plants showing relatively high frequency of dicentric bridges and acentric fragments, occasional double bridges (bridges consisting of two chromatids resulting from four-strand double cross-overs within the inversion) are expected. McCLINTOCK (5) reported a low frequency of such configurations. In this material, one telophase I (fig. 2*F*) clearly showed a double

bridge, a single chromatid bridge, and two fragments. One of the fragments was not in the same plane as the bridges.

Occurrence of micronuclei in quartets.—The frequency of chromosome loss during the two divisions of meiosis can be estimated by the percentage of

TABLE 6

NUMBER OF QUARTETS EXAMINED AND PERCENTAGE OF VARIOUS TYPES OF QUARTETS WITH MICRONUCLEI

Types of quartets	Percentage									
	Orchard grass			Crested wheatgrass				Tall oat grass		
	Plant no.			Plant no.				Plant no.		
	11(13)	22(2)	138(3)	4(1)	4(4)	4(6)	9(1)	4(4)	9(7)	12(3)
1	2.6	6.7	11.1	4.5	.....	12.3	3.5	1.4	3.3	.....
2	1.3	1.5	5.0	4.0	.....	12.3	0.6	2.4	2.6	.....
3	6.0	8.2	12.1	14.8	6.1	23.4	15.6	3.8	5.6	1.5
4	.....	0.5	.....	.....	.....	4.9	.....	1.0	0.3	.....
5	0.4	.....	3.0	.....	.....	6.2	.....	2.8	0.3	.....
6	.....	0.5	.....	.....	.....	.....	.....	1.0	.....	.....
7	.....	.....	.....	.....	.....	.....	.....	.....	0.3	.....
8	0.4	0.5	5.0	.....	.....	4.9	0.6	4.3	0.3	.....
9	0.8	0.5	.....	.....	.....	2.5	.....	0.5	.....	.....
10	.....	.....	1.0	.....	.....	6.2	.....	1.4	0.3	.....
11	0.4	0.5	.....	.....	.....	3.7	.....	0.5	0.3	.....
12	.....	0.5	2.0	.....	.....	1.2	.....	0.5	.....	.....
13	.....	.....	.....	.....	.....	.....	.....	0.5	.....	.....
14	.....	.....	2.0	.....	.....	.....	.....	.....	.....	.....
19	.....	.....	.....	.....	.....	.....	.....	1.0	.....	.....
20	.....	.....	.....	.....	.....	.....	.....	1.0	.....	.....
21	.....	.....	3.0	.....	.....	.....	.....	.....	.....	.....
30	.....	0.5	.....	0.6	.....	.....	.....	.....	.....	.....
31	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....
32	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....
33	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....
34	.....	.....	1.0	.....	.....	.....	.....	.....	.....	.....
No. quartets examined	235	195	99	176	180	81	173	210	306	205
Percentage of quartets showing micronuclei	11.9	19.9	48.2	23.9	6.1	77.6	20.3	22.1	13.3	1.5

immature microspores showing micronuclei. All data on frequency of micronuclei were taken from preparations in which the planes of both meiotic divisions were clearly visible (fig. 1*L*). From table 6 it may be seen that the plants showed wide differences in percentage of quartets, which showed one or more micronuclei in at

least one cell. The origin of these micronuclei is of considerable importance. A comparison of the percentage of quartets showing micronuclei (table 6) with the percentage of sporocytes having lagging univalents at anaphase I (table 3) shows a rather close relationship between these characters. As stated previously, the lagging univalents at anaphase I divided equationally and the two halves were included in the daughter nuclei, or—less frequently—were left in the cytoplasm. Those which were included in the telophase I nuclei would be unable to take a normal part in the second division and might lag and form micronuclei in the microspores. No evidence of degeneration of the micronuclei during interphase I was obtained. If they did not disintegrate, they would persist as micronuclei in the quartets.

TABLE 7

TOTAL NUMBER OF MICRONUCLEI PER 100 QUARTETS EXPECTED ON ASSUMPTION THAT ALL HALF CHROMOSOMES RESULTING FROM LAGGING UNIVALENTS AT ANAPHASE I ARE LEFT IN CYTOPLASM AS MICRONUCLEI; AND NUMBER OF MICRONUCLEI OBTAINED

Plant no.	Orchard grass		Plant no.	Crested wheatgrass		Plant no.	Tall oat grass	
	Expected	Obtained		Expected	Obtained		Expected	Obtained
11(13)	54.6	19.4	4(1)	83.4	34.8	4(4)	59.0	64.2
22(2)	68.7	36.1	4(4)	46.6	6.1	9(7)	70.0	23.7
138(3)	348.0	133.3	4(6)	109.2	168.8	12(3)	10.4	1.5
			9(1)	132.0	25.6			

The fact that the percentage of quartets showing micronuclei was generally lower than the percentage of nuclei showing laggards at anaphase I in a particular plant indicates that the half chromosomes are frequently included in the quartet nuclei. Since each univalent should result in two micronuclei, it should be possible to predict the total number of micronuclei expected per 100 quartets, if none of the half chromosomes are included in the quartet nuclei. These expected values and the numbers obtained are given for each plant in table 7. In eight of the ten plants, the obtained values were considerably lower than the expected, indicating that a majority of the half chromosomes must have been included in the nuclei during the second division. Of the two plants which were exceptions, the data for crested wheatgrass 4(6) may not be reliable since only eleven anaphase I figures were examined. Tall oat grass 4(4) may represent a true exception. The question arises whether micronuclei originate in this material from any source other than lagging and dividing univalents at anaphase I. It seems possible that some could arise from disturbances in the second division itself. If the excess of obtained over expected frequency of micronuclei in tall oat grass 4(4) is sig-

nificant, micronuclei must have arisen in this plant from some other source. No estimate of error is available, however, and the significance of this difference cannot be determined. Some evidence bearing on the question can be derived from the types of quartets formed by the different plants. In all observed cases, the two half chromosomes from a lagging univalent were passing to opposite poles and apparently were destined to be included in different telophase I nuclei or to be left as micronuclei in different cells of the sporocyte. If this is uniformly the case, it is possible to predict the types of quartets, based on the number and position of micronuclei, which can be produced from sporocytes with different numbers of lagging univalents at anaphase I. The expected types from sporocytes with one, two, and three lagging univalents are given in figure 3*A*. If neither half chromosome is included in a nucleus at telophase II, a sporocyte with one laggard would produce either type one or two. If one of the half chromosomes is included in a telophase II nucleus, type three would result. Sporocytes with two lagging univalents can produce types one to twelve inclusive, while those with three can produce types one to thirty inclusive. A plant with a low frequency of sporocytes with two lagging univalents will produce only a low proportion of types four to twelve, and not all these types would be expected unless large numbers of sporocytes were examined. The same condition would obtain for types thirteen to thirty in plants showing a low percentage of anaphase I nuclei with three lagging univalents. Obviously, if all half chromosomes are included in the telophase II nuclei, a normal appearing quartet would result. This would account for the reduction in the percentage of quartets with micronuclei in most plants below the expectation from anaphase I data.

The percentages of quartets of the various types are given in table 6. Tall oat grass 12(3) was the only plant, among those in which a large number of anaphase I figures were examined, which never showed more than one lagging univalent. As expected, this plant had no more complicated types of quartets than type three.

Orchard grass 11(13) and crested wheatgrass 4(4) and 4(6) each had anaphase I figures with one and two lagging univalents. Again, as expected, these plants showed no more complicated types of quartets with micronuclei than type twelve. A maximum of three univalents occurred in the sporocytes studied in orchard grass 22(2), crested wheatgrass 4(1), and tall oat grass 4(4) and 9(7). All except tall oat grass 9(7) had types above twelve but none had types higher than thirty. Only one plant, orchard grass 138(3), had more than three univalents per anaphase I. In this plant, four quartet types (fig. 3*B*) in addition to the thirty obtainable from three univalents were observed. These are numbered 31–34 in table 6. Six lagging univalents would have been necessary to account for type thirty-four. Of the sporocytes examined in this plant, 6.2 per cent of the ana-

phase I figures showed six univalents. These results on types of quartets formed by different plants are in agreement with the premises outlined and indicate that,

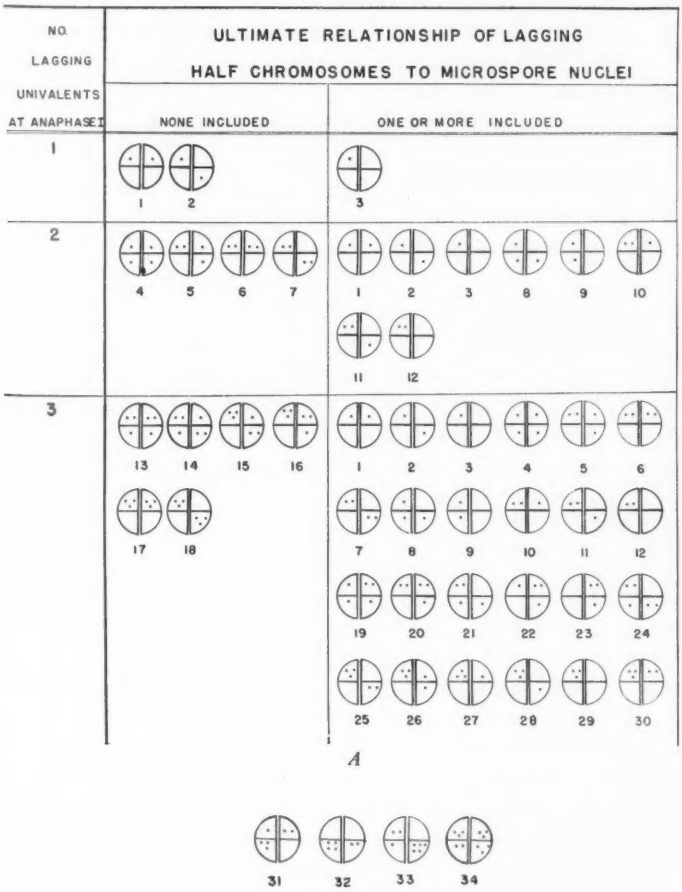

FIG. 3.—*A* (1–30), types of quartets (based on number and position of micronuclei) expected from sporocytes with 1, 2, and 3 lagging univalents at anaphase I, on assumption that micronuclei arise only from loss of half chromosomes derived from lagging univalents; *B* (31–34), four types of quartets obtained in orchard grass 138(3) which are not expected from sporocytes with 1, 2, or 3 lagging univalents at anaphase I.

in this material, most—if not all—of the micronuclei found in the quartet cells resulted from loss of half chromosomes originating from lagging univalents at anaphase I.

Discussion

The frequency of aneuploid plants of orchard grass found in this study is somewhat higher than that reported by MÜNTZING (9), who found 10 per cent on the basis of "accurate values" and 19 per cent on the basis of "total values." It is possible that the difference between MÜNTZING's results and those obtained in this investigation can be accounted for, in part at least, by the parentage of the plants. Four of the progenies studied by MÜNTZING were from single isolated mother plants known to have $2n = 28$. The parentage of the other four progenies was unknown, but the percentage of aberrants in both groups of four was the same. The plants used in the present study came from open pollinated seed stocks and therefore some of the parent plants were probably aneuploid. It seems logical to suppose that the frequency of aneuploidy should be higher among the progeny of aneuploid than of euploid plants. The writers are unaware of previous reports of aneuploid plants in tall oat grass. In crested wheatgrass, PETO (12) reported one plant with $2n = 29$.

The quadrivalent frequencies in the three species investigated were almost the same. The results obtained for orchard grass agreed closely with those reported by MÜNTZING (9), who found an average of 3.8 quadrivalents per sporocyte in polar views of metaphase I in this species. Similar values were found by VON BERG (1) for *Hordeum bulbosum*, by PETO (13) for an induced autotetraploid barley, and by the writers (unpublished) for induced autotetraploids in perennial ryegrass. This similarity in the quadrivalent frequency for the different autotetraploid species of Gramineae with $n = 7$ suggests some common determining factor. The quadrivalent frequencies found in tall oat grass and crested wheatgrass do not agree with those reported by other workers. KATTERMANN (3) found 4 to 7 quadrivalents in tall oat grass and PETO (12), in reporting studies of tetraploid crested wheatgrass, stated that "pollen mother cell preparations on some of this material showed 14 bivalents in the heterotypic metaphase." In the plants studied by the writers, no nuclei with 14 bivalents were observed.

It has been suggested that some species which now behave cytologically as allopolyploids may have been derived from autopolyploids by a process of chromosomal differentiation and selection such that eventually only bivalents are produced at metaphase I. The presence of inversions, indicated by the chromatin bridges and fragments observed in all plants used in this investigation, would be a valuable aid in such a process of differentiation. Inversions have been found generally to cause great reduction in crossing-over throughout the chromosome arms in which they occur. Even if such inhibition of crossing-over in the normal chromosome segments is not universal, very little effective crossing-over within the inverted area would occur, since all single cross-overs would result in deficiencies and would probably therefore be inviable. Such inhibition of recombina-

tions resulting from crossing-over would cause an accumulation of genic and chromosomal changes in the chromosome arm containing the inversion and thus gradually result in differentiation which would reduce homology. The different quadrivalent frequencies shown by the plants investigated suggest that some selection toward bivalent instead of quadrivalent formation may already have occurred, but it does not appear to have proceeded very far. Many plants of these three species, in addition to those reported in this paper, have been examined, and in all cases quadrivalents were found in meiosis. This suggests that selection for reduced quadrivalent frequency has not been an important factor in these species or that the species are relatively young from the standpoint of number of sexual generations. Both factors might be operating. In perennial species, the selection for the higher fertility resulting from more regular meiosis would be less important than in annual types dependent entirely upon seed production for survival. Also perennial species would not be dependent upon a sexual generation each year as are annuals. This may account, in part at least, for the fact that the only species of Gramineae which have been reported to behave cytologically as autopolyploids are perennial in growth habit.

Wide differences were found for the plants studied in each of the meiotic irregularities recorded, namely, univalents at metaphase I, lagging and dividing univalents at anaphase I, and presence of micronuclei at interphase I and in the quartets. The magnitude of the differences suggests that they are significant. Further, the high correlation of these irregularities in the different plants suggests that there is a relationship among them and that the differences are conditioned by heritable factors rather than by environmental fluctuations. POWERS (**14, 15**) found a correlation between meiotic irregularities in varieties of *Triticum aestivum*, and MYERS and POWERS (**11**), using presence of micronuclei in the quartets as a measure of meiotic instability, demonstrated that heritable differences for this character occurred in these varieties.

The data presented in this paper yield some information on the nature of the relationship between the abnormalities studied. The correlation of frequency of univalents at metaphase I with that of lagging univalents at anaphase I indicates that probably most of the unpaired chromosomes lag at the equatorial plate. In some plants there were more lagging univalents at anaphase I than could be accounted for by the number of univalents at metaphase I, indicating their origin from some other source. The lagging univalents divided equationally and the daughter half chromosomes moved toward the two poles, either to be included in the daughter nuclei or left in the cytoplasm as chromatin clumps or micronuclei. The frequency of micronuclei at interphase I was considerably lower than expected if all the half chromosomes were left in the cytoplasm, indicating that a majority were included in the interphase I nuclei. In all but two of the plants

studied, the number of micronuclei in the quartet cells was lower than predicted on the assumption that all lagging and dividing univalents at anaphase I were destined to be left in the cytoplasm at the end of the second division. The observed number of micronuclei varied from 13 to 52 per cent of the expected. The data for one of the exceptional plants were too limited to be reliable. The other may represent a true exception. Whether the half chromosomes are capable of movement at anaphase II, or are merely included in the quartet nuclei if lying close to them, has not been determined. The possibility of origin of micronuclei from some source other than lagging and dividing univalents at anaphase I was suggested in the one plant which had more micronuclei than expected on this assumption. The significance of this excess, however, could not be established from the data available. A comparison of the types of quartets obtained with the types expected on the assumption that micronuclei arise only from dividing univalents indicated that in this material they originate from other sources rarely, if at all.

Aneuploid gametes apparently may be produced either from unequal distribution of quadrivalents or trivalents plus univalents or from the lagging and dividing univalents at anaphase I. For example, if a single univalent lags and divides in the first division and if both half chromosomes form micronuclei, there will be produced two microspores with 13 and two with 14 chromosomes; or, if both are included in the quartet nuclei, one microspore with 13, two with 14, and one with 15 chromosomes will be produced. The wide differences in univalent frequency between the plants studied suggest the possibility of selecting for a low univalent frequency at anaphase I in order to reduce the number of aneuploid gametes.

From the standpoint of practical breeding, these results are of considerable importance. Theoretically plants behaving cytologically as autotetraploids will show tetrasomic inheritance in genetic studies. Preliminary genetical data indicate that tetrasomic ratios are obtained in orchard grass. Tetrasomic inheritance greatly complicates the varietal improvement program in recombination of desirable characters, tests to determine homozygosity of plants for particular characters, or rate of approach to homozygosity in inbreeding. The occurrence of aneuploidy adds further complication, since F_1 plants might be trisomic or pentasomic for the chromosome carrying the particular gene being studied. Corresponding ratios would then be obtained in F_2.

Summary

1. The chromosome numbers of 116 plants of orchard grass were determined from root tips. Twenty-two per cent had 27 chromosomes, 59 per cent 28, 12 per cent 29, and 8 per cent 30. One plant out of ten in tall oat grass had 27

chromosomes, and one out of six in crested wheatgrass had approximately 31 chromosomes, while the rest were normal ($2n = 28$).

2. In all three species the chromosomes were associated in pairs at mid-prophase of the first division, but frequent exchanges of partners indicated some quadrivalent formation. Occasionally a single pair of homologues were associated throughout their entire length.

3. The number of quadrivalents per microsporocyte varied from 1 to 7 in each species. For three plants of orchard grass the range in average quadrivalent frequency was 3.3 to 4.2 with an average of 3.9; for five plants of crested wheatgrass it was 3.4 to 4.2 with an average of 3.7; and for nine plants of tall oat grass it was 3.4 to 4.5 with an average of 3.8.

4. The percentage of ring and chain quadrivalents showing an alternate type of orientation at metaphase I varied from 61.8 to 90.7 among four plants of tall oat grass and was 79.1 and 90.6, respectively, for two plants of crested wheatgrass.

5. The plants of each species varied considerably in percentage of sporocytes with one or more univalents at metaphase I. The lowest frequency was none in 184 sporocytes examined in one plant of tall oat grass, whereas the highest frequency was 32.3 per cent in one plant of crested wheatgrass. The high proportion of sporocytes with two univalents as compared with the number with one and the morphological similarity of the two univalents in most cases suggested that two univalents plus one bivalent were frequently formed instead of one univalent and one trivalent.

6. Lagging univalents at anaphase I divided equationally and the daughter half chromosomes moved toward the two poles. The frequency of lagging univalents in different plants corresponded in general with the frequency of their metaphase I nuclei showing unpaired chromosomes. In most plants there was an excess of laggards, indicating that some originated from other sources than metaphase I univalents.

7. The number of micronuclei in the sporocytes at interphase I was lower than expected from the number of lagging and dividing univalents at anaphase I, suggesting that a majority of the half chromosomes arrived at the poles in time to be included in the daughter nuclei.

8. Chromatin bridges and acentric fragments which apparently had arisen from crossing-over in inversions were found at anaphase I, telophase I, or both stages in all plants studied. Three plants showed one or more sporocytes with two bridges plus two fragments, indicating that each may have been heterozygous for two inversions.

9. The percentage of quartets showing micronuclei was correlated in the different plants with the frequency of sporocytes showing lagging univalents at anaphase I. Observed numbers of micronuclei in the quartets from eight plants

varied from 13 to 52 per cent of the expected numbers, which were calculated on the assumption that all laggards formed micronuclei and none were included in the quartet nuclei. One plant had more than was expected and may have been a valid exception.

10. The number and position of the micronuclei in the quartets confirm the assumption that most, if not all, of them originated from lagging and dividing univalents at anaphase I. Only the types expected on this assumption were observed in any plant.

11. The magnitude of the differences between plants in meiotic irregularities (univalents at metaphase I, lagging and dividing univalents at anaphase I, and micronuclei at interphase I and in the quartets) and the correlation of the frequencies of these irregularities at different stages within the same plant suggest that these differences are significant and probably conditioned by heritable factors.

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FLORAL INITIATION IN BILOXI SOYBEANS AS INFLUENCED BY PHOTOSYNTHETIC ACTIVITY DURING THE INDUCTION PERIOD

M. W. PARKER[1] AND H. A. BORTHWICK[2]

(WITH ONE FIGURE)

Introduction

Previous investigations (1, 4) have shown that photoperiodic induction in two typical short-day plants, Biloxi soybean and *Xanthium*, is dependent upon both light and dark periods of certain lengths. While the exact nature of the reactions affecting induction during either the light or the dark periods is unknown, it has been shown that the soybean requires light of at least 100 foot-candles' intensity if induction is to occur (2). This seems to indicate that in soybeans floral initiation is dependent upon the daily occurrence of a certain amount of photosynthetic activity during induction. The experiments described here were so designed that photosynthesis could be limited and the effect on floral induction observed. This limitation was accomplished in some experiments by removing the carbon dioxide from the air and in others by reducing the amount of light energy.

Apparatus and methods

The apparatus for the experiments involving carbon dioxide was mounted on two warehouse trucks so that it could be readily moved to and from the darkhouse (fig. 1). The plants employed were usually 8–12 inches in height and had three fully expanded trifoliate leaves. They were either grown in or transferred to fern pots 8 inches in diameter and 4 inches deep. The plants were maintained in the greenhouse on 16-hour photoperiods until the beginning of the experiments. These photoperiods consisted of the natural photoperiod extended with Mazda light of 30–40 foot-candles' intensity. In most experiments each pot contained four plants. The plants and the pot were covered with a bell jar and sealed to ground-glass plates with a mixture of modeling clay and castor oil. Air was drawn through the system by means of a vacuum pump. The intake lines were so arranged that either natural air or CO_2-free air could be introduced into the jar. Air from the greenhouse was passed through moist soda-lime towers to remove CO_2. The incoming CO_2-free air stream to each jar passed through a half-saturated solution of barium hydroxide in order to provide a constant check on the efficiency of CO_2 absorption by the soda lime.

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Excessively high temperature inside the bell jars was prevented by the circulation of cold water through small coils of copper tubing. The coils were about 6 inches long and 2 inches in diameter. Their ends extended through the rubber stoppers, which supported the coils in a vertical position immediately below the stoppers. The water was cooled by a portable unit refrigerator and pumped through the coils. By this system the air temperature inside the jars was maintained within about 5° F. of that in the greenhouse, except during brief periods of very bright light, when it rose a few degrees higher. When the apparatus was

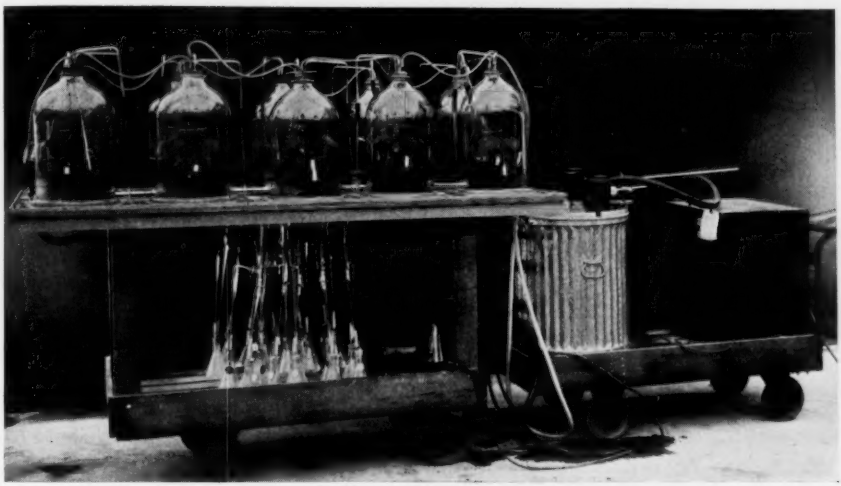

FIG. 1.—Apparatus used in experiments dealing with control of CO_2 in air

moved into the darkhouse, the circulation of cold water was continued for about half an hour and was then automatically stopped. Further cooling was thus prevented after the jars reached the temperature of the darkhouse. Circulation of cold water was resumed when the plants were returned to the greenhouse.

Carbon-dioxide determinations were made with either the Haldane-Henderson new model carbon-dioxide apparatus or the portable Orsat apparatus. The former was used for CO_2 contents of less than 1 per cent and the latter for higher concentrations.

Results

Reduction of floral initiation by decrease of CO_2 supply

A series of four experiments dealing with the effect of reduced photosynthetic activity during induction upon floral initiation was conducted during the fall and winter months of 1939 and the spring months of 1940. Control of photo-

synthesis was obtained by limiting the amount of CO_2 supplied. The induction treatments in the four experiments consisted of six 8-hour photoperiods. The plants were sealed under the bell jars at about 4 P.M. and as usual received supplementary light until midnight. All plants received natural air until 8 A.M. the following morning, which was the beginning of the first 8-hour photoperiod. At this time two of the ten jars were shifted to CO_2-free air just before the trucks were moved from the darkhouse to the greenhouse. These two jars continued receiving CO_2-free air during the entire 6-day induction period. Three pairs of the remaining eight jars were shifted daily from natural air to CO_2-free air at 10 A.M., noon, and 2 P.M., respectively, during the 6-day induction treatment. The reverse shift of the six jars from CO_2-free air to natural air was made at

TABLE 1

PHOTOPERIODIC RESPONSE OF BILOXI SOYBEANS SUBJECTED TO VARIOUS DURATIONS OF NATURAL AND CO_2-FREE AIR DURING 8-HOUR PHOTOPERIODS

Treatment during 8-hour photoperiod		Total plants treated	Total plants bearing flower primordia	Total buds bearing flower primordia per lot	Mean no. of buds bearing flower primordia per flower-bearing plant
Hours natural air	Hours CO_2-free air				
0	8	30	0	0	0.0
2	6	30	6	6	1.0
4	4	30	17	31	1.8
6	2	26	24	58	2.4
8	0	30	24	58	2.4

8 A.M. each day after the experiment was started. The remaining jars were supplied with natural air throughout the 6-day induction period. The five pairs of jars thus received respectively 0, 2, 4, 6, and 8 hours of natural air during each of the daily 8-hour photoperiods. The treatment was continued 6 days. At 8 A.M. of the seventh day the plants were removed from the jars and returned to 16-hour photoperiods in the greenhouse. After approximately 10 days to 2 weeks, the plants were dissected. The results are summarized in table 1.

In these experiments the duration of application of CO_2-free air influenced the flowering response of the plants. When CO_2 was removed from the air during the induction period no floral initiation occurred. Applications of 2 hours of natural air each day resulted in floral initiation in only a few plants, but longer treatments—particularly 6 or 8 hours—resulted in a marked increase, not only in number of plants per lot producing flower primordia but also in number of flower primordia initiated per plant.

In two experiments similar to those reported in table 1, flower primordia were formed on the plants that were scheduled to receive CO_2-free air continuously during the entire induction period. This result was caused by using barium hydroxide of too high concentration in the traps. In these two experiments the air stream to the two bell jars was frequently decreased or stopped during the dark period by accumulation of crystallized barium hydroxide in the line leading through the trap. This permitted the accumulation of CO_2 from respiration of the experimental plants and soil organisms within the jar. Although the air flow was started again before removing the plants from the darkhouse, it is probable that considerable time elapsed before the CO_2 was completely swept out of the jar. The presence of this CO_2 is a possible cause of the formation of flower buds in these lots.

Experiments were accordingly performed to determine whether or not plants receiving no CO_2 from the outside air would be able to initiate flower primordia when subjected to short photoperiods. Plants in fern pots were sealed under bell jars as in the previous experiments. When the plants were placed in the dark at 4 P.M. the flow of air to the jars was stopped by sealing the inlet and outlet lines. At 8 A.M. the plants were moved into the light and left for 2 hours before flow through the jars was resumed. Beginning at 10 A.M., CO_2-free air was again circulated through them until 4 P.M., when they were returned to the dark. After 6 days of such treatment the plants were removed from the jars and grown for several days on 16-hour photoperiods before they were dissected. In each of two experiments of this type the plants produced more flower primordia than did control plants under jars receiving natural air at all times. Other controls receiving CO_2-free air continuously produced no flower primordia.

Since this response was apparently correlated with CO_2 relationship under the jars, similar experiments were performed in which concentrations of the accumulated CO_2 were determined. In these experiments an estimate of the relative amount of CO_2 produced by the soil and its microörganisms and the amount produced by the plants was obtained. The apparatus was set up in the usual way except that pots containing four plants each were placed under some of the bell jars, and pots containing soil only were placed under others. As in the previous experiments, the jars were sealed at 4 P.M. and moved into the darkhouse. At 8 A.M. samples of 100 ml. of air were removed from the various jars, which remained sealed and were moved into the greenhouse at about 8:30 A.M. After 2 hours of illumination a second air sample was taken from each jar. CO_2-free air was then circulated through all the jars until 4 P.M.

At the end of the dark period the CO_2 concentrations in the various jars with plants ranged from 1.5 to 1.2 per cent. The concentrations in the jars containing only pots of soil ranged from 0.7 to 0.4 per cent. The four plants evidently pro-

duced about two-thirds of the accumulated CO_2 and the soil and its organisms one-third.

After 2 hours in the light the CO_2 content of the air over the plants ranged from 0.7 to 0.1 per cent, depending on the intensity of the light. During this period the CO_2 content of the air over the pots containing only soil increased about 0.1 per cent. During the 2 hours of light the plants assimilated an amount of CO_2 approximately equal to that which they respired during the dark period. If circulation of CO_2-free air had been delayed longer than 2 hours the remaining CO_2 would undoubtedly have been assimilated.

The results of these experiments show that stoppage of the CO_2-free air line during the dark period will allow the accumulation of sufficient CO_2 to cause initiation.

Reduction of floral initiation by decrease of light energy

In a series of two experiments, photosynthesis was limited by reducing the amount of light energy supplied to the plants, and its effect on floral initiation was determined. The results from previous experiments of this type (**2**) have shown that the extent of initiation is correlated with the amount of light energy received by the plants. In those experiments, however, the light energy was limited by reducing the intensity; in the present experiments it was limited by reducing the duration of exposure to light. This latter method made the results more directly comparable with those of the previous experiments, which dealt with reduced CO_2 during the induction period.

Experiments involving decreased light energy were conducted in the greenhouse with daylight as the high-intensity source. Although the intensity of natural light fluctuated from hour to hour, it was sufficient at all times to insure active photosynthesis and was far in excess of the intensity of artificial light previously used in similar experiments (**2**). In the two experiments summarized in table 2, photoperiods of eight types were used. These types differed in their relative numbers of hours of high and low intensity light but were all 8 hours in duration. The high-intensity portion of these photoperiods ranged from 1 to 8 hours per day. In all cases where durations of high intensity shorter than 8 hours were used the photoperiods were extended to 8 hours with Mazda light of 10–20 foot-candles. By this intensity the dark periods to which all lots were subjected were made identical. The additional light used to bring this about was below the threshold intensity (**2**) previously found effective in causing initiation and constituted in all cases a very small part of the total light received by the plants.

The high-intensity part of all photoperiods was begun at 8 A.M. each day by moving the various lots from the darkhouse to the greenhouse. At hourly intervals, beginning at 9 A.M., lots were returned to the darkhouse, where they were

subjected to about 10 foot-candles of Mazda light until 4 P.M. At this time the final lot was placed in the darkhouse and the Mazda light was turned off until the following morning.

In each of the experiments the various photoperiodic treatments were applied on four successive days in one series of lots and six successive days in another series. Each lot consisted of 20 plants, making a total of 160 plants per series, or 320 per experiment. The data were subjected to variance analysis, and both photoperiod and duration of treatment were found to be significant at odds of more than 99 to 1. Since there was no significant interaction between the two factors, only the totals based on the summations of both lots have been included in table 2.

TABLE 2

INFLUENCE UPON FLORAL INITIATION OF VARIOUS AMOUNTS OF LIGHT ENERGY RECEIVED BY BILOXI SOYBEANS DURING 8-HOUR PHOTOPERIODS

Treatment during 8-hour photoperiod		Total plants bearing flower primordia per lot of 80	Total buds bearing flower primordia per lot	Mean no. of buds bearing flower primordia per flower-bearing plant	Mean no. of nodes in main axis per plant
Hours of high-intensity natural light	Hours of low-intensity Mazda light				
1	7	0	0	0.0	19.1
2	6	10	13	1.3	19.7
3	5	62	104	1.7	19.7
4	4	75	205	2.7	20.1
5	3	77	282	3.7	20.2
6	2	80	313	3.9	20.4
7	1	80	351	4.4	20.6
8	0	80	395	4.9	20.9

The total number of plants per lot that formed flower primordia in response to these photoperiodic treatments is correlated directly with the number of hours of high-intensity light included in the 8-hour photoperiod. When the photoperiod consisted of 6 hours or more, all plants initiated flower primordia. With 3 hours of high intensity daily, about 75 per cent of the plants formed flower primordia, and with shorter durations of high intensity the number of plants that initiated flower buds decreased very abruptly. In the lots that received 3, 4, and 5 hours of high-intensity light daily most of the plants that failed to initiate flower primordia were those that received only 4 days of treatment. This might suggest that, had treatments with 1 and 2 hours of high-intensity light been applied more than 6 days, flower-bud formation would have been more abundant. The appearance of the plants in these two lots at the end of the 6 days showed that they

would not have survived treatments of much longer duration. Apparently they required more than 1 hour per day to maintain themselves. With 1 hour per day the stems elongated excessively and the leaves lost color. Each additional day of treatment served further to weaken the plants.

The number of buds per lot containing flower primordia and the mean number per plant on those that initiated flower buds were correlated with the number of hours of high-intensity light. Each additional hour of light from 1 to 8 hours caused an increase in number of flower buds. This was true even among those lots receiving 6, 7, and 8 hours of high-intensity light in which all plants initiated flower primordia.

Since the buds containing flower primordia occur singly at certain nodes of the main axis, it might be assumed that their number would be influenced by the rate of formation of new nodes in the main axis. The data of table 2 show that there is a progressive increase in number of nodes with increase in the total amount of light applied. This difference was less than two nodes, however, while the difference in number of buds containing flower primordia was 4.9. The production of buds containing flower primordia was evidently not limited by the rate of production of new nodes.

Increase of floral initiation by short daily applications of air enriched with CO_2

Since it was possible to limit induction by reducing photosynthetic activity with the two methods applied, some stimulation to induction might be expected if photosynthesis could be accelerated. Such acceleration was attempted in a series of three experiments by increasing the concentration of CO_2 in natural air. Four different concentrations of CO_2 were established in the various bell jars each morning during the treatment period, before bringing the plants into the light. These higher concentrations were obtained by adding definite amounts of pure CO_2 to the partially evacuated chambers. The remaining vacuum was released by allowing natural air to flow into the jars. The inlet and outlet air lines to each jar were then closed and the apparatus was moved into the greenhouse. After remaining for 2 hours in the light, the air lines were again opened and CO_2-free air was circulated through each jar during the remainder of the photoperiod and until 8 A.M. the next day.

In this series of experiments, $\frac{1}{2}$, 1, 2, and 4 liters of CO_2 were introduced into four different chambers. These amounts of CO_2 made the concentrations within the jars approximately 2.5, 5, 10, and 20 per cent, respectively, as measured by the Orsat apparatus. Variation in the volume of the jars as well as that of the pots and plants produced minor differences in these concentrations. In each experiment two chambers received natural air and two received CO_2-free air during

the photoperiod. The results of the three experiments are summarized in series 1, table 3.

When plants received natural air during the photoperiod they formed an average of 2.5 flower primordia per plant, and only two of the twenty-five plants failed to produce any flower buds. Only one plant of the nineteen receiving CO_2-free air initiated any flower buds, however, and this plant had flower buds at only

TABLE 3

EFFECT ON FLORAL INITIATION IN BILOXI SOYBEANS OF CO_2 CONCENTRATIONS GREATER THAN FOUND IN NATURAL AIR

Composition of air in bell jars	Percentage concentration of CO_2 in enriched air at beginning of treatment	Duration (in hours) of treatment in CO_2 enriched air*	Total plants treated	Total plants bearing flower primordia	Total buds bearing flower primordia per lot	Mean no. of buds bearing flower primordia per flower-bearing plant
	Series 1					
CO_2-free			19	1	1	1.0
Natural			25	23	58	2.5
Enriched with CO_2	2.5	2	12	12	51	4.2
	5.0	2	12	12	54	4.5
	10.0	2	12	12	50	4.2
	20.0	2	12	9	37	4.1
	Series 2					
CO_2-free			7	0	0	0.0
Natural			8	7	13	1.8
Enriched with CO_2	5.0	½	8	7	18	2.6
	5.0	1	7	7	21	3.0
	5.0	2	8	8	33	4.1
	5.0	3	8	8	38	4.7
	20.0	½	8	8	23	2.8
	20.0	1	8	8	25	3.1
	20.0	2	8	8	28	3.5
	20.0	3	8	8	29	3.6

* Plants received CO_2-free air during remainder of each 8-hour photoperiod and accompanying dark period.

one node. When the air was enriched to 2.5, 5, or 10 per cent CO_2, all the plants initiated flower buds and formed many more than were formed on plants receiving natural air in the jars. When 20 per cent CO_2 was used, however, only nine of the twelve plants treated formed flower primordia, but these nine formed nearly as many flower buds per plant as when lower concentrations were used.

Since no appreciable differences in floral initiation occurred when several concentrations of CO_2 were supplied for 2 hours, treatments with concentrations of 5 and 20 per cent CO_2 were applied for ½, 1, 2, and 3 hours. By varying the dura-

tion of treatment in this manner a correlation between the amount of CO_2 received and the extent of initiation was shown. A summary of the results from two such experiments is shown in series 2, table 3. In both experiments one lot of plants received natural air and one received CO_2-free air during the photoperiod. All the plants that received air enriched with CO_2 during part of the photoperiod were given CO_2-free air during the remainder of each photoperiod and the following dark period.

The air that was enriched to 5 per cent CO_2 had a greater effect on induction than air that was enriched to 20 per cent CO_2. This difference was not present in the $\frac{1}{2}$- and 1-hour treatments but became conspicuous as the duration was extended to 2 or 3 hours. In another experiment in which 20 per cent CO_2 was used for periods longer than 3 hours, definite injury to the plants resulted.

Table 3 shows that increased concentration of CO_2 in the bell jars caused an increase in floral initiation. Of the concentrations used, even the lower ones were very effective. Thus a 5 per cent concentration supplied for only $\frac{1}{2}$ hour per day, or a 2.5 per cent concentration supplied for 2 hours per day, resulted in the formation of more primordia than were formed on controls receiving natural air continuously.

These results also offer a possible explanation of why more flower primordia were formed on plants that had been sealed in bell jars during the dark period and for the first 2 hours of the photoperiod than on plants that had received natural air in the jars at all times. The concentrations of CO_2 that accumulated under those conditions were found to be 1.5 to 1.2 per cent. While these concentrations were lower than those reported in table 3, they were probably sufficient to stimulate the production of more flower primordia than were formed on controls receiving natural air.

In several experiments plants receiving natural air under bell jars were compared with similar plants receiving natural air in the greenhouse without bell jars. In all such cases the latter plants produced many more flower primordia than the former, although the photoperiodic treatments were otherwise identical. Factors that could account for these differences include reduction of the light intensity, higher temperature, and a possible deficiency in CO_2 under the jars.

To eliminate the possibility of CO_2 deficiency, bell jars were suspended over several series of plants so that the bottom of the jar extended down only to the top of the pots. The top opening was covered with a layer of cheesecloth. This arrangement allowed greenhouse air to circulate freely through the jars. The controls for these experiments consisted of similar plants over which no jars were suspended. In a series of four experiments a total of 79 plants had bell jars suspended over them and 79 others served as controls. These plants were given six 8-hour photoperiods, after which the jars were removed and the plants returned

to 16-hour photoperiods. After 2 weeks the plants were dissected. Of the 79 plants that had been under bell jars, 61 formed flower primordia in a total of 194 buds, or in 3.2 buds per plant. All the 79 control plants initiated flower buds in a total of 415 buds, or in 5.2 buds per plant. This formation of many more flower buds on the plants outside the bell jar was characteristic of each of the four experiments. Evidently the reduced light or higher temperature or a combination of both under the jars reduced the flower-forming stimulus.

The possibility that CO_2 may also have been limiting because of inadequate rate of air flow was investigated in another series of experiments by studying the CO_2 content of air entering and leaving the bell jars at different rates. Flowmeters were used to determine these rates, and samples for CO_2 analysis were obtained in sampling tubes inserted in intake and exhaust lines. To insure uniform samples, the tubes were left in the line 5 minutes, at which time they were simultaneously removed and the gas immediately analyzed. When air was flowing through the jars at the rate of 45 liters per hour the CO_2 content on a bright sunny day decreased from 0.03 to less than 0.01 per cent. At a rate of 60 liters per hour the exhaust air contained on the average about 0.02 per cent CO_2. When a rate of 100 liters per hour was used only slight changes in the CO_2 content of air were detected. The results indicate that under the conditions of these experiments the CO_2 content of natural air is not greatly depleted if flows of 60 or more liters per hour are maintained. In all the experiments reported herein, flows of 60 or more liters of air per hour were maintained. The reduction in floral initiation under bell jars seems to be caused by factors such as reduced light intensity and higher temperature, rather than by deficiency of CO_2 as supplied by natural air. The number of flowers formed on plants receiving increased CO_2 supply under bell jars appears therefore to be a resultant of the stimulating effect of increased CO_2 concentration and the retarding effect of the jars themselves.

Increase of floral initiation by continuous application of air enriched with CO_2

In the experiments dealing with short daily applications of CO_2 the treated plants in all cases produced more flower primordia than the control plants under bell jars receiving natural air. In a few of these experiments an additional lot of control plants not under jars was included. These plants received the same photoperiodic treatment as the other controls. Although many more flower primordia were formed in all cases on plants receiving CO_2 than on controls receiving natural air under the bell jars, the most favorable CO_2 treatments induced no more flower buds than were formed on the controls that were not under the jars. It seemed probable, however, that with a more favorable duration and method of application of CO_2 it should be possible to initiate more primordia on plants under the

jars than on those outside. To do this the apparatus previously used was modified so that a constant stream of air enriched with CO_2 to any desired percentage could be supplied to the plants.

Four-day induction treatments were given instead of the usual 6-day periods, so that fewer flower primordia would be initiated on the controls. If the plants had received 6-day treatments it would have been possible under optimum conditions that all the meristems capable of being induced on the controls would have formed primordia. By the use of a shortened induction period, any experimental treatment that was more favorable to floral initiation than that imposed on the controls should therefore result in flower-bud formation at these additional points, and the effectiveness of such treatments could be measured.

TABLE 4

EFFECT ON INDUCTION IN BILOXI SOYBEANS OF CONTINUOUS SUPPLY OF AIR ENRICHED TO 1 PER CENT CO_2

TREATMENT	TOTAL PLANTS TREATED	TOTAL PLANTS BEARING FLOWER PRIMORDIA	TOTAL BUDS BEARING FLOWER PRIMORDIA PER LOT	MEAN NO. OF BUDS BEARING FLOWER PRIMORDIA PER FLOWER-BEARING PLANT	MEAN NO. OF NODES PER PLANT
Control in greenhouse	60	56	176	3.1	17.8
Natural air through bell jar at 60 liters per hour	60	51	98	1.9	17.3
Air enriched to 1% CO_2 through bell jar at 60 liters per hour	60	60	201	3.3	18.2

In these experiments five of the bell jars received natural air at a rate of 60 liters per hour and the other five received air enriched to approximately 1 per cent CO_2 at 60 liters per hour. At the end of the 4-day induction period the plants were returned to 16-hour photoperiods. Two weeks later they were dissected.

In all the experiments, the plants that received air enriched with CO_2 to 1 per cent had more flower primordia than the controls that were not under the bell jars (table 4). Each of these lots had more primordia than the lot under jars receiving natural air. By enriching the air with CO_2 it was possible to cause more flower primordia to be initiated on plants under the jars than on the controls. This stimulative effect of CO_2 on initiation must have been really greater than the results indicated, since it has been shown that the jars themselves have an inhibitory effect on initiation.

Discussion

The data of previous papers (**4, 6**) have indicated that in Biloxi soybean reactions during the light period and others during the dark period are both essential

to floral initiation. In the present paper data are presented showing that the effectiveness of the photoperiodic treatment in causing initiation is correlated with the amount of CO_2 assimilated by the plants during the light period. The initiation of flower primordia in Biloxi soybean was limited in one case by reducing the amount of CO_2 made available to the plants during induction and in another by reducing the total amount of light supplied. Both treatments resulted in limiting the amount of CO_2 that the plants assimilate. Conversely the initiation of flower primordia was increased by increasing the concentration of CO_2 in the atmosphere surrounding the plants. When conditions of light and CO_2 supply are such that considerable photosynthetic activity occurs each day during the induction period, more flower buds are initiated than when less daily photosynthetic activity occurs.

Active photosynthesis is apparently important in the photoperiodic reaction through its effect expressed within each individual leaf, rather than through its effect on the plant as a whole. Evidence for this was found in three experiments, each of which by a different method restricted the amount of photosynthesis per plant during induction. In one experiment photosynthesis was reduced by defoliation (3), in another by limitation of CO_2, and in the third by reduction in total amount of light energy supplied. When photosynthesis was limited during the induction period by these various methods, the number of flower primordia formed per plant was found to depend upon the method used. When the leaf area of a plant was reduced to about one-fourth or less by removal of all but one of the leaves, the number of flower primordia formed in response to photoperiodic treatment was frequently as great as when all leaves were present (3). In this experiment the amount of photosynthesis per plant was greatly reduced, but the amount per leaf was probably about the same as in the undefoliated controls. If, however, the amount of photosynthesis per plant was reduced by limiting the amount of light energy or of CO_2 available to the plant, the initiation of flower primordia was greatly reduced. In this case the products of photosynthesis per plant may have been about the same as in the previously described defoliated plants, but the amount per leaf was probably much lower. This seems to indicate that the individual leaves must accumulate an amount of photosynthate beyond a certain threshold value before reactions will proceed that ultimately result in initiation.

It is generally accepted that the reactions responsible for floral initiation arise in the leaf (2, 5). Evidently the products of such reactions migrate from the leaves to the growing points, and there cause the formation of flower primordia. These flower-forming substances may be either immediate products of photosynthesis or products derived from them. In either event it should make no difference whether they were supplied to the growing points in small amounts by several leaves or in

larger amounts by one leaf. Since flower-bud formation occurs more abundantly in the latter case, the terminal must be receiving more flower-forming substances. The production of these substances must therefore depend on an adequate supply of the products of photosynthesis in the individual leaf and must arise through their transformation.

Summary

1. Initiation of flower primordia in Biloxi soybean was limited by controlling photosynthesis during induction. This was done in one case by controlling the CO_2 supplied to the plants and in another by controlling the duration of high-intensity light.

2. When no CO_2 was supplied during 8-hour photoperiods, no initiation of primordia occurred. Plants that received the CO_2 contained in natural air during 2, 4, 6, and 8 hours of each photoperiod produced flower primordia in proportion to the duration of time the natural air was supplied.

3. Plants that received only 1 hour of high-intensity light during an 8-hour photoperiod formed no flower primordia. Those that received 2 hours or more of high-intensity light during the photoperiod produced increasingly larger numbers of flower primordia as the duration of high-intensity light increased.

4. Increased concentration of CO_2 in natural air resulted in increased floral initiation.

The writers wish to acknowledge the assistance of Dr. P. H. HEINZE, who made the gas analyses reported in this paper.

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LENGTH OF DAY AND TEMPERATURE EFFECTS IN RUDBECKIA[1]

A. E. MURNEEK

(WITH ELEVEN FIGURES)

Introduction

The two major aspects of photoperiodism in plants would seem to be the modification in size and form of vegetative development and the acceleration or delay of sexual reproduction. While both of these general effects of length of day have been widely observed and described by GARNER and ALLARD (**5, 6**) and subsequently by many other investigators of photoperiodism, none seem to have studied more closely the relationship, or absence of it, between photoperiodic inhibition and photoperiodic induction (for terminology see **11**).

A previous paper (**10**) has shown that the influence of length of day on stem elongation and on flowering are two separate phenomena, although brought about by the same causal external factors (**1**). By a certain combination of photoperiods, *Rudbeckia* plants of either natural size or with extremely reduced or inhibited stem development (rosettes) were made to produce flowers normally. The present paper deals with a further study of photoperiodic inhibition and induction in *R. bicolor* var. *superba* and *R. speciosa* Newmanii (Wenderath), the latter species being used in a general way only.

Material and procedure

Under ordinary greenhouse conditions the seeds of *Rudbeckia* germinate promptly, the seedlings are easy to transplant, and they grow slowly. This comparative sluggishness in their development makes them less sensitive to minor alterations in the external factors, which is often highly desirable when the greenhouse environment is only partly under control.

Seeds were secured from a reliable commercial source, and the germinal variability within the species was slight. During the 2 years that this investigation was in progress the treatment of the seedlings and mature plants was identical. They were grown in good garden soil in 5-inch clay pots.

Plants exposed to short days were given 7 hours of light between 8:00 A.M. and 3:00 P.M., while those receiving long days were given 14 hours of light, when necessary the daylight being extended by supplementary electric lighting of about

[1] Aided in part by a grant from the Rockefeller Foundation. Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station, Journal Series no. 661.

250 foot-candles. The general greenhouse equipment has been described elsewhere (11). In almost all instances the experimental treatments were repeated; hence the results are drawn from duplicate groups of plants grown in different years.

Results

Behavior under natural short and long days

When grown at an average day temperature of 70°–75° F. and approximately 5°–10° lower at night, under a natural length of day between November and July, at Columbia, Missouri (10.5 to 14+ hours of photoperiod), *Rudbeckia* plants behaved as follows. The seedlings developed slowly during the short days of the late fall and winter months into an extremely flat rosette until about the middle of March, when the daylight had lengthened to 12 hours. The leaves then assumed a more vertical position (fig. 1), which seems to be the first sign of change in development—most probably induction of the reproductive phase. This was followed about 2 weeks later by rapid elongation of the main stem and axillary shoots, until in late spring a height of 85–95 cm. was attained, when the plants were in full bloom (fig. 2, left). The critical light period (photoperiod) for initiation of reproduction in *R. bicolor* is therefore approximately 12 hours or slightly more (17). It may be the same for *R. speciosa*, although this has not been definitely established.

If from the seedling stage on, the plants are exposed continuously to long photoperiod (14-hour day), development is quite similar. Naturally the rosette stage is then comparatively brief or almost absent. While long days remarkably hasten development from seed to seed, the final height and form are the same as when plants are grown under natural length of day. *Rudbeckia* is a long-day plant. When exposed continuously to a relatively short photoperiod of less than 12 hours, the plants remain in the rosette stage indefinitely (fig. 2, right), some having been maintained in this form for more than a year. Neither do they grow in height nor become reproductive, provided the temperature is not extreme. At any time, however, they may be made to develop normally by moving them to long-day exposure, photoperiodic induction beginning in every case on the second day, as indicated by change in position of leaves. Retardation in development is not permanent, therefore, no matter how long continued.

In general, a relatively short photoperiod of less than 12 hours inhibits both reproduction and stem elongation in *Rudbeckia*, and a photoperiod exceeding 12 hours leads to induction of reproduction, followed shortly by growth in height of the plant.

Photoperiodic induction and inhibition

While it has previously been demonstrated in a preliminary way (10) that in *Rudbeckia* the photoperiod controls separately sexual reproduction and vegetative

growth, more extensive evidence seemed desirable as final proof of the independence of the two major effects produced by the light period.

FIGS. 1–4.—*R. bicolor*. Fig. 1, effects of length of day on position of leaves: left, grown under photoperiod < 12 hours; right, under photoperiod > 12 hours. Erect position seems the first sign of induction of reproduction. Fig. 2, left, grown under long (14-hour) day; right, under short (7-hour) day. Fig. 3, right to left: plants having received preliminary long-day exposure of 20, 25, 30, 35, and 40 days respectively, when moved (January 23) to short-day exposure were promptly inhibited in growth in height and looked like this a month later (February 23). Fig. 4, plants having received long-day induction of 35 days (left) and 40 days (right), respectively, inhibited in growth in height and developed some flowers normally, others subnormally or abnormally (vegetatively).

Uniform plants of *R. bicolor* that had been exposed in groups to a 14-hour day, at 5-day intervals for 20–40 days, were switched to short-day exposure (on Jan-

uary 23). Because of the preliminary long-day treatment, photoperiodic induction to various degrees had been brought about in these plants, although as yet there were no visible flower buds. The plants varied in height from 2 to 20 cm. but were extremely uniform in this respect within each subgroup (table 1, group 1).

TABLE 1

PHOTOPERIODIC INHIBITION IN R. BICOLOR: DEMONSTRATION BY MEANS OF SWITCHOVER EFFECTS FROM LONG- TO SHORT-DAY EXPOSURES

GROUP 1: LONG → SHORT DAYS, ON JANUARY 23

PRELIMINARY EXPOSURE		HEIGHT OF PLANTS (CM.), LONG→SHORT			HEIGHT OF PLANTS (CM.), LONG CONTINUOUS (CHECKS)	
BEGINNING OF LONG-DAY EXPOSURE	NO. OF DAYS UNDER LONG PHOTOPERIOD (TO JAN. 23)	JAN 23, DAY OF SWITCHOVER	FEB. 25	APR. 6	JAN. 23	APR. 6
Dec. 13	40	20	20	21	20	90
Dec. 18	35	15	15	16	15	90
Dec. 23	30	8	8	8	8	90
Dec. 29	25	3	3	3	3	90
Jan. 3	20	2	2	3	2	90

GROUP 2: LONG→SHORT DAYS, ON MARCH 1

PRELIMINARY EXPOSURE		HEIGHT OF PLANTS (CM.), LONG→SHORT			HEIGHT OF PLANTS (CM.), LONG CONTINUOUS (CHECKS)		
BEGINNING OF LONG-DAY EXPOSURE	NO. OF DAYS UNDER LONG PHOTOPERIOD (TO MAR. 1)	MAR. 1, DAY OF SWITCHOVER	MAR. 25	APR. 16	MAR. 1	MAR. 25	APR. 16
Jan. 27	31	34	36	36	34	80	90
Feb. 2	26	23	24	24	23	63	90
Feb. 7	21	11	11	11	11	50	90

As early as 2 days after they had been subjected to short photoperiod, plants of the December 29 and January 3 subgroups had turned their leaves into a more horizontal position, while those receiving a more extended preliminary long-day treatment did not exhibit this movement. But the most striking effect of the long → short-day plants was their prompt inhibition of growth in height. On February 25, a month after they had been switched, the height was still the same, and on April 6, when most of them were beginning to bloom, it had not noticeably changed.

While their height did not alter under short photoperiod, additional leaves

were produced in abundance, indicating that all axillary shoots had formed rosettes of foliage, giving an extremely bunched appearance to the plants (figs. 3–5). The usually long pedicels of the flowers were also mostly (but not always) abbreviated (fig. 4). Plants receiving the minimal preliminary long-day treatment (20 or 25 days) produced either one sessile flower or none at all (fig. 6), and such flowers were usually abnormal. Evidently photoperiodic induction of

FIGS. 5, 6.—*R. bicolor*. Fig. 5, vegetative flowers and vegetative rosettes on partly induced plants. Fig. 6, plants that received minimal long-day exposure (20–25 days) either did not flower (left) or produced one sessile flower (right) on short photoperiod.

reproduction in these cases was either partial or incomplete, and if complete, further development of flower parts was greatly retarded or inhibited. There were often highly "vegetative" flowers or strictly vegetative foliage rosettes present (fig. 5). The check plants, kept continuously under long photoperiods from the respective days of beginning of long-day exposure (December 13 to January 3), although varying in height considerably at the beginning (2–20 cm.), in due time grew to the same height, reaching their final growth of 90 cm. and becoming fully and normally reproductive (fig. 7).

Another group of plants was given the same experimental treatment in late

spring, when the greenhouse temperature was higher. The results were essentially the same (table 1, group 2). The slight increase in height of 1–2 cm. in some cases

FIGS. 7–9.—*R. bicolor*. Fig. 7, although varying in height (2–20 cm.) when moved to long-day exposure, plants eventually attained same stature and uniform development. Fig. 8, flowering brought about under short (7-hour) photoperiod by relatively high temperature. Fig. 9, high temperature may induce flowering under short days, with frequent stretching of pedicels.

may have been brought about through secondary stimulation at the time of flower development (**9, 11, 12**), or it may have been due to an expected lag during the period of adjustment from long to short photoperiods.

Effects of high temperature

Not being provided with refrigeration, the temperature in the greenhouse—despite proper ventilation—frequently became very high during the summer months. When the day temperature had been rising for a protracted period to 90°–100° F., occasionally higher, and the nights were proportionally warm, an unexpected thing happened. The short-day rosette type plants, which had been con-

Figs. 10, 11.—Fig. 10, vegetative flowers of *R. bicolor* produced as result of exposure on short photoperiod to relatively high temperature (constant at 90° F.). Fig. 11, *R. bicolor* plants 250 days old (left), grown continuously under short photoperiod, came into bloom as result of high temperature. *R. speciosa* plants 250 days old (right) did not flower under same treatment.

tinuously and still were under a 7-hour photoperiod, began to bloom. In all instances only one central flower was produced, but it was usually normal in size and fully developed. Although most of the pedicels of these flowers were short (fig. 8), some had stretched and thereby elevated a few of the bractlike leaves (fig. 9), possibly due to stimulation by the flower itself (**9, 12**). In several cases the flowers were extremely large or highly vegetative (fig. 10). After considerable time a vegetative rosette-like flower would occasionally change into a usually subnormal flower with petals and reproductive organs. Otherwise these short-day

high-temperature plants remained unchanged in their rosette form, except for the typically more erect position of the leaves. Under long photoperiod, *R. bicolor* plants grew tall and developed normally. These results were obtained during two summers and therefore may be considered typical.

To check somewhat more closely the temperature factor, groups of plants were exposed in midwinter, when the days are naturally short, to constant temperatures of 50°, 70°, and 90° F.[2] After 2 days' sojourn in this uniform environment, plants in the 90° chamber had their leaves lifted and, although not growing in height, became sexually reproductive early in the spring. At uniform temperature of 70° there was also some indication of flower development, but none at 50° F. Unfortunately these artificial conditions did not imitate the fluctuating night and day temperatures of natural summer weather. It suggests, however, that probably it is the relatively high night temperature that is conducive to sexual reproduction (but not growth) of *R. bicolor*.

Although *R. speciosa* is also a long-day plant and in general responds to photoperiods of 14 and 7 hours' duration, very much like *R. bicolor*, it does not seem to be similarly sensitive to high temperature. When the two species are grown side by side on short photoperiod, they remain indefinitely in the rosette state of development, but the former does not bloom under a high temperature in June and July while the latter produces flowers (fig. 11). It would seem that *R. bicolor*, a more southern species, is "attuned" in respect to sexual reproduction to both relatively long days and high temperature, while *R. speciosa*, a more northern species, is influenced in this respect only by long photoperiod and apparently not by high temperature.

Discussion

The present study has demonstrated that—in some plants at least—the influence of length of day on vegetative growth and on reproduction are separate responses, and that temperature may replace the photoperiod in induction of sexual reproduction.

The conclusion from the results with *R. bicolor* would seem to be as follows: (*a*) Growth in height is fostered and maintained, but not induced, under long-day exposure; hence when plants are placed on short photoperiod, growth of the stem and branches ceases. (*b*) Reproduction is not only brought about by long days but also induced. Photoperiodic induction having taken place, flower development will continue although the plants be placed under short-day exposure—a light environment nonconducive to sexual reproduction in this species. (*c*) Photoperiodic induction and photoperiodic inhibition, two separate responses, can be combined in various ways by exposure to appropriate lengths of day.

[2] The special temperature-control greenhouse was made available through the courtesy of Dr. E. BROWN of the Field Crops Department, University of Missouri.

Why should reproduction be induced but not growth when *R. bicolor* plants are exposed to relatively long days? A definite answer to this question may be made only when more accurate knowledge of the physiological causes responsible for initiation of reproduction and for maintenance of vegetative development is available. It is possible that in specific plants the formation of growth substances is fostered by certain photoperiods and not by others, and that this is a day-to-day affair with no accumulation of reserves. Hence when plants are switched to an opposite photoperiod, a shortage ensues and growth stops promptly. Or have we to deal here with inhibitors of types suggested in recent botanical literature?

That reproduction may be induced by exposure of plants to very few appropriate photoperiods has been demonstrated repeatedly. When the exposure is minimal, but few floral primordia may be able to develop into flower buds and still fewer into flowers of normal size and function. Accordingly, when *Rudbeckia* plants, after a few days' exposure to long photoperiod, are switched to short days, the flowers are subnormal in size and form, or of so-called vegetative type (**2**). This would seem to indicate a quantitative relationship between the production of the flower hormone, whatever its nature, and flower development (**10**).

The modifying effects of temperature on photoperiodism and the direct influence of temperature on sexual reproduction have been known for some time (**5, 6, 7, 18, 14, 8**). In respect to initiation of flowers, some plants may be very sensitive to heat or cold (**18, 4**). The results obtained with *R. bicolor* would seem to indicate that, as regards sexual reproduction, this species is "attuned" not only to relatively long days but also to relatively high temperature, so that the latter, as an environmental factor, may be substituted for the photoperiod. This is in agreement with previous more general observations that flowering and fruiting in this species are favored by a combination of long days and warm temperature (**17**). It is probable that comparatively warm nights instead of warm days may be more effective in this respect (**3**), and that—with normal fluctuations of temperature—there may well be certain favorable or unfavorable relationships between relatively high and low temperatures (**15**).

That in respect to flowering *R. speciosa* is not so sensitive to high temperature is probably due to its more northern habitat. Apparently through natural selection, based on segregation of characters or mutations, these two species have become adjusted to different environments, while other species or varieties of *Rudbeckia* may show further variability in this respect. Horticultural types, as for example a double-flower form of *R. laciniata*, may be desirable mutations, selected not only because of type of flower but also general or specific adaptability to light, temperature, and possibly other environmental factors (**16**). Their responses to light and heat may be different. Specific and varietal variability of this nature undoubtedly exists in almost any group of cultivated plants.

It would seem needless to emphasize the fact that great variability exists among plants in respect to their response to relatively long and short photoperiods. The cultivated dill (*Anethum graveolens* Linn.), which the writer has studied, although appearing to be a long-day plant is really an "indeterminate" or "neutral" one. The retarded growth in height and apparent vegetativeness under short-day (7-hour) exposure is merely delay in development. In a short photoperiod it required approximately 3 months for our plants to reach full stature and anthesis, while in a long photoperiod the same height and stage of development was attained in a little over 1 month. Similarly when short-day dill plants were exposed in graded series to 1–12 long days and then returned to a short photoperiod, their subsequent speed of development was in proportion to the number of long days (1–12) received. Therefore, in reference to temporarily reduced growth of dill plants under short-day exposure, it is not permissible to speak of "photoperiodic inhibition" but only of retarded development.

Summary

1. Growth in height occurs and sexual reproduction is initiated and maintained in *Rudbeckia bicolor* under a photoperiod exceeding 12 hours. In short photoperiod, less than 12 hours, the plants remain in a rosette type of development (photoperiodic inhibition) and do not produce flowers.

2. Reproduction may be induced (photoperiodic induction) to various extents by exposure to a certain number of long days. Floral organs continue to develop and may function normally when plants are placed under short-day treatment.

3. Stem elongation cannot be induced by but only maintained on long photoperiods. Growth in height stops promptly when *R. bicolor* plants are switched from long to short photoperiod.

4. Photoperiodic induction and photoperiodic inhibition may be combined in various ways by exposing plants to appropriate combinations of long and short days. Thereby the size and form of the vegetative parts and the amount and character of development of reproductive tissues may be changed. Vegetative flowers and leaf rosettes are produced.

5. High temperature may substitute for long days in induction of reproduction in *R. bicolor*, a more southern species, but not in *R. speciosa*, a more northern one. Stem elongation is not fostered under short days by increase in temperature.

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PARTHENOCARPIC AND NORMAL FRUITS COMPARED AS TO PERCENTAGE OF SETTING AND SIZE[1]

FELIX G. GUSTAFSON

(WITH ONE FIGURE)

Introduction

In the work on artificial parthenocarpy two questions have repeatedly been asked. Are the fruits produced artificially as large as those produced by pollination? And how does the percentage of setting compare with pollination?

In the early experiments (3) there was no accurate record kept of the number of flowers treated, nor were enough fruits measured to give accurate information as to their relative size. In the paper on parthenocarpy in holly, GARDNER and MARTH (2) state: "To all outward appearances the parthenocarpic fruits are like those developed following pollination, with the exception that, in the case of naphthalene acetic acid, the fruits continue to be much greener in color and somewhat larger in size." Their illustrations confirm this statement. In 1938, the writer published some results (4) on the rate of growth and final size of tobacco fruits when produced by potassium indoleacetate and by pollination. It was found that during the first week of development the parthenocarpic fruits grew faster than the normal ones, but the final size of the seeded fruits was greater than that of the parthenocarpic fruits. WONG (11) has also found that parthenocarpic watermelons are smaller than the seeded ones.

In an experiment with cucumbers grown in the greenhouse in the spring of 1937, it was found that the setting with 5 per cent indoleacetic acid in lanolin was 28 per cent, while the setting produced by hand pollination was 66.6 per cent. The diameter of the former fruits was 12.2 cm. and the length 32.8 cm., while the diameter of the latter was 14.9 cm. and the length 37.8 cm. The percentage of setting and the size of the fruits are thus seen to be greater when produced by hand pollination.

Investigation

In the summer of 1938 several hundred tomato plants were grown in heavily fertilized soil at the Arcadia Farm belonging to the California Institute of Technology, Pasadena, California. John Baer tomatoes were chosen because this is the plant with which the writer has had the best success in producing parthenocarpic fruits.

[1] Paper from the Department of Botany and the Botanic Garden of the University of Michigan, no. 716.

Owing to the very heavy growth, it was impossible to record all fruits that were set from the different treatments, and no information was gained on the number that set. To determine the rate of growth and final size, 31 fruits produced by natural pollination, 53 fruits produced by phenylacetic acid, and 15 produced by indolebutyric acid treatment were tagged and measured at frequent intervals until the fruits were ripe or had reached a stage where there was no further increase in size. Two diameters at right angles to each other and at right angles to the axis passing through the stem of the fruit were measured.

Figure 1 shows that the rate of growth and the final size reached were almost identical for the fruits produced by pollination and by phenylacetic acid. At the beginning of the measurement the indolebutyric acid-produced fruits were somewhat larger and the final size was also a little larger than the others.

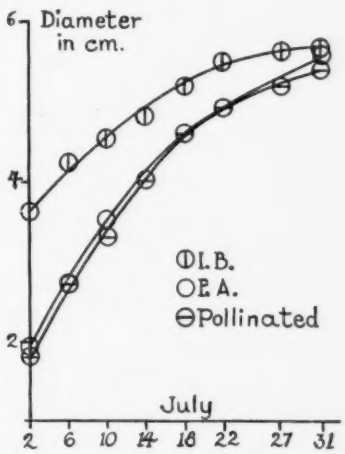

FIG. 1.—Progressive enlargement of tomato fruits produced by treating the ovaries with indolebutyric or phenylacetic acids or by pollination.

During the summer of 1939 a similar experiment was conducted at Ann Arbor, Michigan. The soil was not so rich as that near Pasadena. This time the plants were pruned to a single stem and staked so that it was a simple matter to find all the treated flowers or fruits. Three different-aged plants were used in order to determine whether age and vigor had any influence upon the setting and size of fruits produced. A fourth lot of plants was grown from seeds that had been soaked in a 0.4 per cent solution of colchicine for 3 days just before planting, and a few flowers from a commercially grown lot of the same variety of plants were also treated at the time those plants were loaded with fruits.

As is usual with this variety when grown in the field, pollen was not easy to obtain and only fifteen flowers were pollinated on July 13 and 16, all on plants of lot 2. Of these, twelve, or 80 per cent, produced fruits. On July 13, when only the first two lots of plants were in blossom, flowers were treated with 3 per cent indoleacetic and indolebutyric acids in lanolin. The results are shown in tables 1 and 2.

By July 16 all four lots of plants were in blossom and a number of flowers were treated with 3 per cent naphthaleneacetic acid. The results are given in table 2. It will be noticed from these two tables that the setting due to chemical treatment of flowers on plants of lot 2 was 90 per cent for indoleacetic, 80 per cent for

indolebutyric, and 100 and 80 per cent for naphthaleneacetic acid. This is a little better than for pollination, which was 80 per cent.

In some of the early experiments on induced parthenocarpy it had been observed that the fruit induction by chemicals was easier to obtain in the first cluster on a plant than in later clusters, after the plants had already produced some fruits. In the John Baer tomato, fruits are not readily produced in the first cluster even when hand pollinated with pollen from the same or other plants. This may indicate that there was present in the young plant a large amount of a substance necessary for the production of parthenocarpic fruits and that this substance decreased as the plants aged or produced other fruits.

GUSTAFSON and HOUGHTALING (**6**) have shown that when tomato vines are deflorated for part of the season they produce more flowers per cluster and the

TABLE 1

SIZE AND PERCENTAGE OF SETTING OF FRUITS FROM TREATED FLOWERS, JULY 13, 1939

CHEMICAL	NO. OF FRUITS PRODUCED	LOT	PERCENTAGE SETTING	DIAMETER (CM.)
3% Indoleacetic.....	9	2	90	4.9
3% Indolebutyric.....	4	1	80	5.93
3% Indolebutyric.....	8	2	80	6.56

TABLE 2

SIZE AND PERCENTAGE OF SETTING OF FRUITS FROM FLOWERS TREATED WITH 3 PER CENT NAPHTHALENEACETIC ACID

DATE OF TREATMENT	NO. OF FRUITS	LOT	PERCENTAGE SETTING	DIAMETER (CM.)
7/16.....	27	1	67.5	5.44
	19	1	0*	5.97
7/13.....	5	1	46.0	5.73
	27	1	0*	6.25
	4	2	100.0	4.57
	38	2	0*	6.25
7/16.....	12	2	80.0	5.28
	8	3	80.0	6.64
	9	4	70.0	6.45
	9	4	0*	7.12

* These fruits were seeded and had been produced by natural pollination; they are used here for the sake of comparison as to size.

fruits produced are much larger than those produced by plants that have been bearing during the whole season. Their interpretation is that the fruits depleted the plant's food supply and the fruits produced later in the season were small, while the fruits produced at the same time on the plants that had been deflorated were large because there had been no depletion of food substances.

These two facts made it desirable to compare old plants that had produced no fruits (due to defloration) and young plants that were just coming into flower. Plants (designated as *b* in table 3) from lots 1 and 2 were therefore deflorated for several weeks after the other plants began to bear.

TABLE 3

SIZE AND PERCENTAGE OF SETTING OF TOMATO FRUITS FROM FLOWERS TREATED WITH 1 PER CENT NAPHTHALENEACETIC ACID, JULY 26, 1939

No. of fruits	Lot	Percentage setting	Diameter (cm.)
6	1*a*	25	4.47
9	1*b*	80	5.07
14	1*b*	0*	5.82
2	2*a*	20	5.15
4	2*b*	56	6.16
16	2*b*	0*	6.24
10	3	66	5.75
5	3	0*	5.34
9	4	43	6.49

* These fruits were seeded and had been produced by natural pollination; they are used here for the sake of comparison as to size. The *a* plants were setting fruits all the time; the *b* plants were not permitted to set until this date.

On July 26 the ovaries of the flowers from the *b* plants—as well as from all the other plants—were treated with 1 per cent naphthaleneacetic acid. Unfortunately lots 3 and 4 had come into blossom by July 16 and were now producing blossoms in the second or third cluster; but by comparing the *b* plants from table 3 with the plants from lots 3 and 4 in table 2, comparable flowers and fruits are obtained. It will be seen that plants 1*b* set 80 per cent on July 26, so did the plants of lot 3, and those from lot 4 set 70 per cent on July 16. Plants 2*b* set only 56 per cent on July 26 while corresponding *a* plants had set 80 per cent on July 16. If a comparison is made between the *a* plants (those that had been bearing all season) and the *b* plants from the corresponding lots, on July 26, it will be seen that the percentage setting is considerably lower in the *a* plants, owing no doubt to depletion of food material as a result of fruit production. Comparing the *b* plants and those from lots 3 and 4, on July 16, it is clear that the percentage of setting in the older plants (that have not been producing any fruits) is nearly as large as in the young plants producing fruits for the first time; and the old plants

are just as capable of producing parthenocarpic fruits as the young plants if they have not been bearing too heavily. Commercially grown plants that had been bearing heavily all summer produced no fruits at all when treated with chemicals on July 26. Evidently production of fruits parthenocarpically does not depend upon the age of the plant but upon its nutritional level, which is dependent upon the amount of fruit previously produced by the plant, and upon its vigor.

In tables 2 and 3 are included fruits produced by open pollination. While the time of setting is not known, the fact that they were ripe and on the same plants

TABLE 4

COMPARISON AS TO SIZE BETWEEN TOMATO FRUITS PRODUCED BY CHEMICALS AND POLLINATION, WHEN GROWN IN SAME CLUSTER

Date of treatment	Parthenocarpic					Seeded	
	Chemical and percentage		Lot	No. of fruits	Size	No. of fruits	Size
7/13	Indolebutyric	3	1	3	5.86	3	6.54
7/16	Naphthaleneacetic	3	1	27	5.75	3	6.11
7/26	Naphthaleneacetic	1	1	12	5.07	8	6.23
7/29	Indolebutyric	1	1	3	5.96	3	6.82
7/13	Indoleacetic	3	2	7	4.93	6	5.63
7/13	Indolebutyric	3	2	8	6.13	8	6.24
7/16	Naphthaleneacetic	3	2	15	5.16	13	5.97
7/29	Indolebutyric	1	2	2	6.56	2	6.07
7/16	Naphthaleneacetic	3	3	7	6.87	7	7.48
7/16	Naphthaleneacetic	3	4	6	6.55	6	7.16
All naphthaleneacetic		3	.....	55	5.81	56	6.36
All naphthaleneacetic		1	.....	12	5.07	8	6.23
All indolebutyric		3	.....	11	6.06	11	6.31

as the ripe parthenocarpic fruits justifies a comparison of their size with that of the parthenocarpic fruits. The fruits produced by pollination are larger in all instances, except on lot 3 treated with 1 per cent naphthaleneacetic acid on July 26, which are somewhat larger than the corresponding seeded fruits.

To make the comparison more definite, the parthenocarpic and seeded fruits which grew in the same cluster have been brought together in table 4. There is only one instance in which the parthenocarpic fruits are larger than the corresponding seeded ones, and here only two fruits are concerned. So far as the John Baer tomato grown in Ann Arbor, Michigan is concerned, therefore, the parthenocarpic fruits are somewhat smaller than the seeded ones. The difference in

size is not great, and undoubtedly the seedless fruits with the somewhat greater amount of pulp per fruit are to be preferred even though a little smaller. On the other hand, figure 1 shows that fruits produced by indolebutyric and phenylacetic acids at Pasadena, California, are as large if not larger than fruits produced by pollination. How can these contradictory results be reconciled? No doubt Californians would say it is due to the climate, but it is more likely due to the heavy fertilization of the soil, which resulted in greater vigor of the plants. The soil in which these plants grew was heavily fertilized with chicken manure, rich in nitrogen, just before the plants were set out. AVERY, BURKHOLDER, and CREIGHTON (1) have shown that the growth hormone content of a plant is influenced by the mineral nutrition. Lack of nitrogen especially decreases the auxin content very much. It is therefore likely that the auxin content of these tomato plants was so great that neither it nor the food substances needed for fruit growth was the limiting factor, while the plants growing in a less fertile soil may be lacking both in food material and auxin. Here the fruits with more auxin might conceivably grow larger.

The smaller size of the parthenocarpic fruits cannot be explained on the basis of difference in the size of the original ovary, as they all come from the same cluster. HOUGHTALING (7) has shown that in several varieties of tomato there was no cell division in the ovaries after anthesis; and MACARTHUR and BUTTLER (8) state: "The post-anthesis period is characterized chiefly by cell expansion, cell division being a minor factor which just suffices to maintain the epidermis, the cells of which do not expand."

The difference in size must therefore be due to the formation of smaller cells in the parthenocarpic fruits than in the seeded fruits. The smaller cell size in the parthenocarpic fruits is undoubtedly due to a lower concentration of auxin, as the food supply is the same. The writer (5) has shown that seeded tomatoes have a greater auxin concentration than have parthenocarpic fruits; the seeds are very rich in auxin. If quantity of auxin is responsible for size, and these experiments point in that direction, it would seem possible to increase the size of the parthenocarpic fruits by supplying them with more auxin. Methods for doing this will have to be developed, as it is not sufficient merely to smear more lanolin paste on the fruits.

These and other experiments on parthenocarpy indicate that the growth of fruits as a result of chemical treatment is in no way different from that taking place as a result of pollination, except that ovules do not develop. The matter of food supply is just as important in the production of both types of fruits. This is to be expected if we consider that an important function of the auxin is to mobilize the food material to the point where growth takes place, as seems to be implied by the experiments of MITCHELL and MARTIN (9) and STUART (10).

Summary

1. The experiments, although rather limited on this point, show that the percentage of setting with chemical treatment is probably as great as with pollination. This refers only to the tomato. On the other hand, the parthenocarpic fruits are definitely smaller than the seeded fruits.

2. The early rate of growth may perhaps be a little greater in the ovaries treated with chemicals than in those pollinated, but this early rate decreases after a while, perhaps due to a lowering of the auxin concentration in them.

The writer wishes to express his appreciation to F. W. WENT, E. G. ANDERSON, J. VAN OVERBEEK, and WM. S. STEWART of the California Institute of Technology, Pasadena, California, for various courtesies and to the Directors of the Horace H. Rackham Trust Fund of the University of Michigan for financial aid.

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POLYPLOID COTTONS OBTAINED THROUGH USE OF COLCHICINE. I. CYTOLOGICAL OBSERVATIONS IN OCTOPLOID GOSSYPIUM HIRSUTUM[1]

A. J. T. MENDES

(WITH TWENTY FIGURES)

Introduction

A preliminary account of the occurrence of polyploid cottons obtained through the use of colchicine has been presented before the First South American Botanical Congress (**5**). Individuals having $2n = 52$ and $2n = 104$ were obtained after treatment of seeds of *Gossypium herbaceum* L. ($2n = 26$) and *G. hirsutum* L. ($2n = 52$), respectively. The treatment which furnished the highest percentage of "duplicated" plants consisted in the immersion of delinted seeds in a 0.15 per cent colchicine solution for about 16 hours. Various treatments, however, such as with a solution of 0.075 per cent for 25 hours or 0.3 per cent for 48 hours, also produced polyploid plants.

The seeds of *G. hirsutum* were from a multiplication field of a commercial variety, while the seeds of *G. herbaceum* were from plants which had been selfed for many generations. While from the latter seeds uniformly abnormal plants were obtained as a result of certain treatment, several different types of abnormalities were found among the plants derived from the seeds of *G. hirsutum*. Some of the seeds did not react at all to the drug. The chromosome numbers were then determined in root tips when the plants were only $5\frac{1}{2}$ months old; at that time they had not yet flowered.

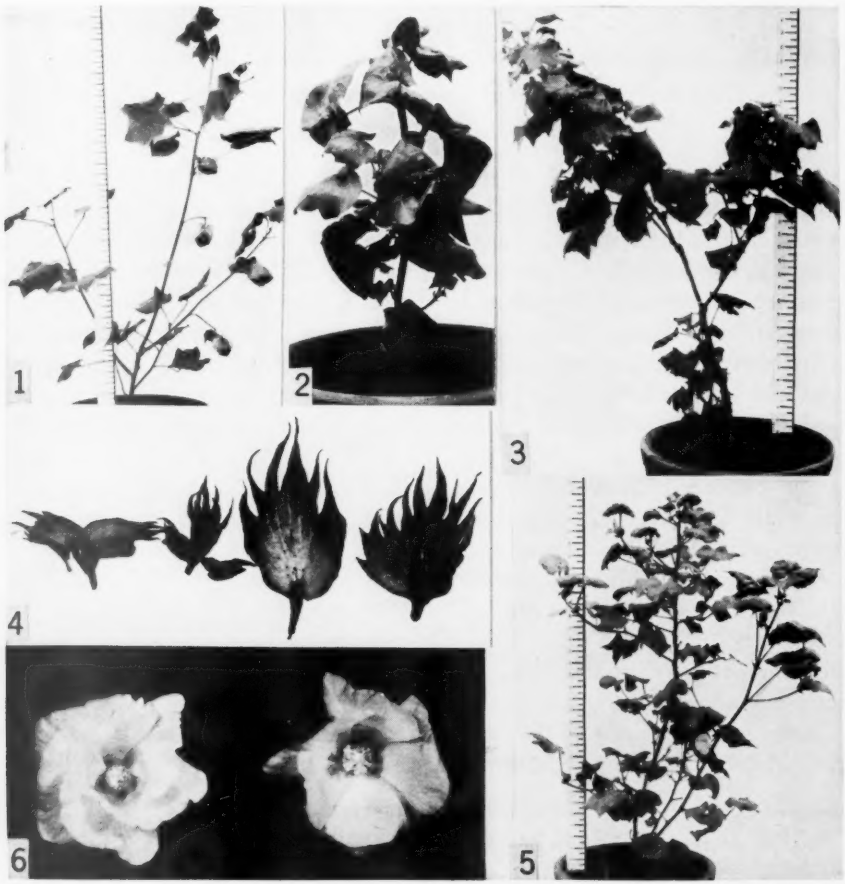
In the present paper only the experiments with *G. hirsutum* (fig. 1) will be presented, leaving for future publication the results obtained with *G. herbaceum*.

Morphological observations

The seeds treated with low percentage (0.06) colchicine solution for a relatively long time (16 hours) did not produce other than normal plants. Seeds treated with high percentage (0.6) solution for a short time (4 hours) produced highly abnormal plants, many of them dying in the cotyledonary stage. Their hypocotyls were greatly swollen and the roots poorly developed; in the root tips, octoploid cells ($2n = 104$) were found. By using a moderately concentrated colchicine solution (0.15 per cent) for 16 hours both normal and abnormal seedlings were obtained; among the abnormals, several soon died. This treatment, however, has been found

[1] Publication authorized by the Director of the Institute as Technical Bulletin no. 76.

to be the most effective in producing entirely octoploid plants. Both severe and moderate treatments can produce entirely octoploid plants, apparently without chimaeras; but the octoploid plants obtained through moderate treatment have a



FIGS. 1–6.—*Gossypium hirsutum*. Fig. 1, $2n = 52$, normal plant. Fig. 2, $2n = 104$, from heavily treated seed; no flowers. Fig. 3, same, from heavily treated seed; flowers reduced to bracts. Fig. 4, abnormal "flowers" from plant shown in fig. 3. Fig. 5, $2n = 104$, from treated seed. Fig. 6, from two plants having $2n = 104$ and $2n = 52$; note indehiscence of anthers in 104-chromosome flower at left.

better chance to survive than plants produced from severely treated seeds, their development also being much more regular.

Several types of abnormalities are found among the plants from severely treated

seeds which do not occur among the others. For instance, a severely treated seed produced an octoploid dwarfed plant ($2n = 104$ in root tips) on which the leaves were entirely different from those of all the other plants, and on which not a single flower bud has yet developed—the plant is now more than 13 months old (fig. 2). Another interesting octoploid plant (fig. 3) had its floral parts reduced to a single bract, with a reduced calyx at its base (fig. 4). The plants which appeared to be tetraploid were eliminated and about seventy of those which seemed to be octoploid were raised to maturity. Plants which proved to be entirely octoploid were rare. It was found that many of the root tips were composed of a mixture of tissues having 52 and 104 chromosomes. Later, while studying microsporogenesis, tetraploid and octoploid cells sometimes were noted in the same flower.

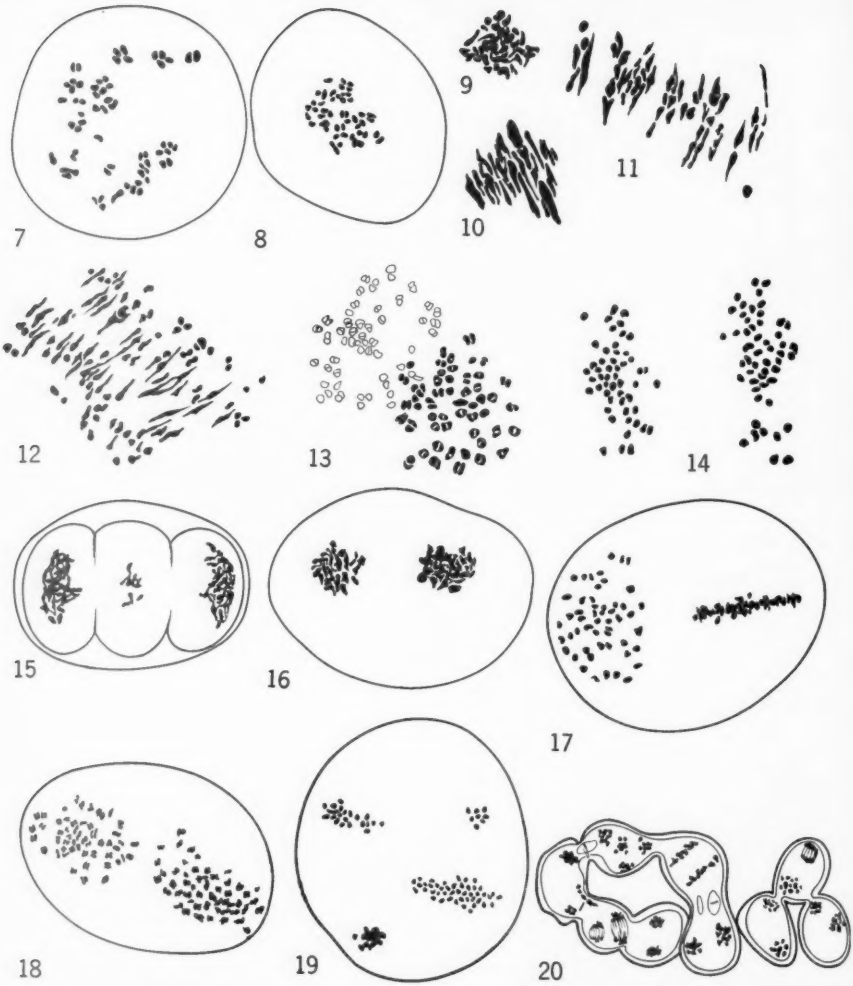
Various characters help to identify adult octoploid plants: coarse leaves of a deeper green color than in normal plants, abnormal branching and stunted growth (fig. 5), and flowers which do not open easily and have thicker petals and indehiscent anthers (fig. 6). The size of the leaves was very variable amongst the octoploids, some of them being tiny and others extremely large.

Cytological observations

An analysis of chromosome distribution in the pollen mother cells of the octoploid plants has been made. Flower buds were collected and fixed in a mixture of 3 parts alcohol to 1 part acetic acid. After 24 hours they were transferred to 70 per cent alcohol and the anthers examined by the aceto-carmine method. All drawings, with exception of figure 20, were made with a camera lucida having a 10× ocular and a 90× objective, giving a magnification of 1070 diameters.

The prophase is difficult to observe in detail. In the thread stage the chromosomes can be seen only when the coverslip is not pressed against the material, but as soon as the coverslip is pressed down the filaments become less visible in the stained plasm. A single large nucleolus is seen in the prophase for a long time. Buds of several sizes show only prophasic cells and the next phases occur in rapid succession. In the same anther may be found cells in every stage of meiotic divisions, from prophase I to young "quartets."

The formation of quadrivalents, the separation of the halves of the bivalents, and a considerable amount of secondary association make the counting of the chromosomes difficult at metaphase I. Figure 7 shows cells in the prometaphase stage having some bivalents free and others associated in groups of two, four, and more. In figure 8 still closer association is observed. This may become so intense that massing of the chromosomes occurs (fig. 9). Close to cells like the one shown in figure 10 are others like that illustrated in figure 11, where the chromosome number could be more easily determined. The difference in the intensity of mass-



FIGS. 7–20.—*Gossypium hirsutum*, $2n=104$. Fig. 7, secondary association in pollen mother cells. Fig. 8, closer association of chromosomes. Fig. 9, massing of chromosomes in pollen mother cells. Fig. 10 ($2n=104$?), profile view of metaphase I. Figs. 11, 12, same of metaphase I. Fig. 13, polar view of anaphase I; chromosomes already divided. Fig. 14, late anaphase I; 52 chromosomes at each pole. Fig. 15, cytokinesis after first division. Fig. 16, massing of chromosomes after first division. Fig. 17, metaphase II; 52 chromosomes in each plate. Fig. 18, metaphase II; chromosomes appear double. Fig. 19, multipolar arrangement of chromosomes. Fig. 20 ($2n=204$, or mixoploid), syncytia of pollen mother cells.

ing at these stages may be caused by various degrees of secondary association at earlier stages.

As already stated, the counting of the chromosomes in polar views of metaphase I is difficult. The bipartition of many chromosomes and the union of others in pairs or larger groups cause confusion; moreover, the anaphasic separation does not occur at the same time for all the chromosomes, so that univalents are often scattered away from the equatorial plate. A profile view of anaphase I is shown in figure 12, where it can be seen that some chromosomes go to the poles earlier than the others. Other anaphases were found in which the separation of the chromosomes occurred regularly and at the same time for all the chromosomes. In late anaphase almost all the chromosomes are already divided (fig. 13).

Many anaphases were observed in detail, and almost always 52 chromosomes were seen to go to each pole (fig. 14). A few pollen mother cells among normal ones from the same anther showed several types of abnormality, such as unequal distribution and losses of chromosomes between the two poles, cytokinesis after the first division, etc. This last type of abnormality has been observed only once and was a very peculiar case, the cytoplasm appearing to be divided into three portions (fig. 15).

At the end of the first anaphase a massing of the chromosomes, identical to the one observed before, can be seen (fig. 16). It seems, however, that the more general rule is the immediate formation of two second equatorial plates (figs. 17, 18). When neither massing nor immediate arrangement of the chromosomes in the metaphasic plates takes place after the first anaphase, interkinetic nuclei are formed, the chromosomes losing their compactness. Chromosomes can be easily counted at metaphase II. As in anaphase I, 52 chromosomes are distributed to each pole in the second division. Almost all the anaphases II are regular; several lost chromosomes which are occasionally observed at this stage (fig. 19) are usually laggards remaining from the first division. As a result of the predominant regularity observed in these divisions, about 73 per cent of all quartets formed really have four cells; only about 15 per cent of the microsporocytes form quartets with five cells and 12 per cent with six cells. Very seldom quartets are formed with still more microspores.

A peculiar type of abnormality, that is, the arrangement of the pollen mother cells in syncytia (fig. 20), already observed by LEVAN (3) in octoploid species of *Petunia*, was also noticed in some *Gossypium* plants. In some cases it was possible to determine their mixoploid nature, but in others it seemed certain that they were entirely octoploid. It was difficult to determine whether the syncytia were formed by tetraploid or by octoploid cells. It appeared evident that anastomosis of the cells occurred during the prophase of the first division. The two mitoses went on regularly in most cells, forming four normal telophasic nuclei, although in some

cases a shifting of chromatic matter from one cell to its neighbor cell was noticed. It is possible that this shifting occurred as a consequence of the pressure on the coverslip.

Pollen, seed, and progeny

As already stated, indehiscence of the anthers was one characteristic of the octoploid plants. This fact is responsible for their high sterility, since the pistil always appeared to be normal and since microsporogenesis was almost completely normal. Occasionally, however, a few anthers in a flower were dehiscent. Studying the size of the pollen grains, it has been found that some anthers were diploid while others were tetraploid. The pollen grains of the untreated plants ($2n = 52$) had a diameter of $106.5 \pm 5.6\ \mu$, while the ones of the treated plants ($2n = 104$) were generally larger and more variable, $138.5 \pm 12.2\ \mu$. In the tetraploids the coefficient of variability was 5.5 per cent, compared with 8.8 per cent in the octoploids.

Self- and cross-pollination, using pollen from normal and treated plants (octoploid or not), has been carried out artificially, but not a single mature fruit has been obtained from more than one hundred flowers. The artificially pollinated flowers produce a fruit that does not develop very far, falling off 3–12 days after pollination. Grafts of the octoploid plants have been made on normal tetraploid ones; here also no fruit set has been obtained. Occasionally a few bolls were produced from open pollinated flowers. These rare fruits differed from the normal ones in being more nearly spherical, smaller, and few-seeded. While in the untreated plants the seeds were uniformly small and long, weighing on the average 0.73 gm., the seeds obtained from the treated plants varied in form, size, and weight. In most cases they were similar to the normal ones in form and size, weighing 0.71–0.87 gm.; in other instances the seeds were almost spherical and much larger, weighing 0.93–1.22 gm.

Some of the seeds were sown in petri dishes and after germination transferred to small pots. At the beginning only a low percentage of the larger seeds germinated, while almost all the normal ones germinated rapidly. Increased germination was later obtained by cutting off the tip of the seed coat (1). All the plants obtained were normal in appearance, flowering, and fruit set. In many of them the chromosome number was $2n = 52$.

Discussion

Specialists (**2, 4, 6, 7**) in the genus *Gossypium* have suggested that a duplication of the chromosomes of ancestral types produced the American cottons with $2n = 52$. These are considered to be tetraploids and the plants having $2n = 104$ are thus octoploids. The secondary association of the chromosomes found in the octoploids is similar to that occurring in the tetraploids. The abnormalities found

during microsporogenesis in octoploids are slightly more pronounced than the ones reported by other workers for the tetraploids. Assuming that the same abnormalities occur at megasporogenesis as has been found at microsporogenesis, they cannot be responsible for the extremely low fruit set of the octoploids, since most of the egg cells should function normally. The high sterility of the octoploids must be due mostly to indehiscence of the anthers and other physiological disturbances.

The highly abnormal plants obtained after heavy colchicine treatments suggest changes in the structure of chromosomes or gene mutations, in addition to duplication of chromosome numbers. The facts that several seeds have been produced by the octoploid plants and that they have developed into normal tetraploid individuals might be explained either through parthenogenetic development of 52-chromosome egg cells or through the fusion of two 26-chromosome gametes both derived from tetraploid tissue islands. The second hypothesis is more probable, since such tetraploid tissue islands have actually been found both in anthers and root tips.

The different shape, the larger size, and consequently the increased weight of the seeds are to be interpreted as results of the influence of the octoploid mother plant. The gigantism of the vegetative organs, found in other known polyploids, has not been found in octoploid *Gossypium*.

Summary

1. Octoploid and tetraploid plants were obtained from seeds of *Gossypium hirsutum* and *G. herbaceum* treated with colchicine.

2. The seeds of *G. herbaceum* were generally homozygous and reacted as a rule more uniformly to the drug than did the seeds of *G. hirsutum*.

3. Microsporogenesis in octoploid ($2n = 104$) *G. hirsutum* is described in some detail: a few chromosomal irregularities were observed and the pollen grains are larger than in the normal tetraploid plants. In spite of the fact that empty pollen grains were rarely found, the plants are highly sterile. The anthers are almost always indehiscent.

4. Flowers with dehiscent anthers were self-pollinated, and others were cross-pollinated with pollen from tetraploid and octoploid plants. No fruit set occurred.

5. A few fruits from open-pollinated flowers have been obtained. Their seeds were in some cases almost spherical and were larger and heavier than the normal. The seed coat was very hard and the percentage of germination low. These seeds yielded normal tetraploids ($2n = 52$).

The writer wishes to express his indebtedness to Dr. L. W. Sharp and Mr. C. A. Krug for helpful suggestions and criticism, and final reading of the manuscript.

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FORMATION OF THE SEED OF CYPRIPEDIUM PARVIFLORUM

MARGERY C. CARLSON

(WITH NINE FIGURES)

Introduction

This study was undertaken to learn the steps in the formation of the seed of the orchid, *Cypripedium parviflorum* Salisb., to obtain a better understanding of the structure of the mature seed, and to obtain, if possible, some clue to the problems involved in the germination of orchid seeds in general. The history of megagametophytes and embryos in *Orchis pallens* was traced by STRASBURGER (16) and in *Gymnadenia conopsea* by WARD (19). TREUB (17) and DUMÉE (5) studied embryos of orchids, especially to determine the types of suspensors present. They found unorganized embryos in all the species studied; single-celled suspensors, which sometimes cannot be distinguished from the embryo proper, in some species; and elongated, multicellular suspensors which extend out through the micropyle in other species. BEER (1) examined mature fruits and seeds of many species of tropical orchids, and CURTISS (4) described seeds of twenty-five species native to the United States and determined their average dimensions and the dimensions of the cells of the seed coats. LEAVITT (6) described variations in suspensors and listed cases of polyembryony in several species of native orchids. VESQUE (18), PACE (9, 10), BROWN (2), BROWN and SHARP (3), and SHARP (13, 14) traced megasporogenesis, development of the megagametophyte, and fertilization in certain species of orchids; but none of these investigators followed the history of the seed beyond the first few divisions of the fertilized egg. PFITZER (11) summarized the information on seeds of orchids available in 1889; NETOLITZKY (8) described the structure of mature seeds; and MAHESHWARI (7) and SCHNARF (15) reviewed the work on megagametophytes, endosperm, and embryos of orchids.

Methods

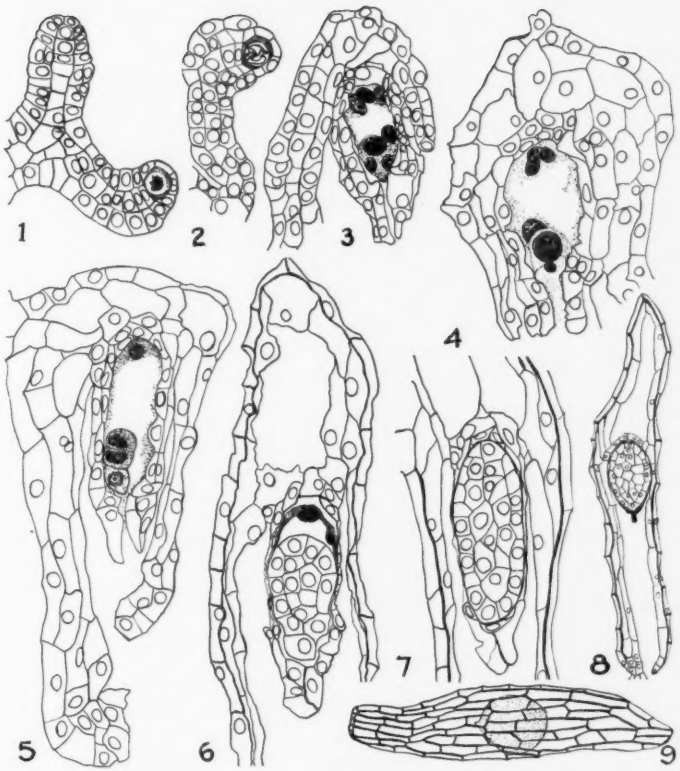
Young flower buds of *Cypripedium parviflorum* were covered with cheesecloth bags until the flowers had opened and their stigmas were receptive. The flowers were then cross-pollinated and rebagged. Pollinations were made on May 24, 28, and on June 5, 1936. Ovaries were fixed in Flemming's and Navashin's solutions just before pollination, and 2, 4, 5, 12, 14, 16, 26, 33, 55, and 72 days after pollination. The material was imbedded in paraffin; sections were cut 10 to 12 μ in thickness and stained in safranin and light green.

Observations

Ovule and integuments

The ovary has a single cavity, with three parietal, forked placentae. In the ovaries of the earliest collections the ovules appear as small, hemispherical, or slightly oval protrusions, formed by rapidly dividing cells of the surface of the placentae. Each ovule primordium consists of a peripheral layer inclosing a single axial row of cells. The outermost cell of the axial row, the archesporial cell, enlarges and functions as the spore mother cell. The ovules increase in length and, in ovaries fixed 2 days after pollination, appear as in figure 1. At this time the spore mother cells have increased in size and have entered the early prophase stages of reduction division, and the first periclinal divisions leading to the formation of the inner integuments have occurred. By the fourth or fifth day following pollination (fig. 2) the stalks of the ovules have lengthened, chiefly by cell divisions, and the tips of the ovules have bent slightly to one side. The lobes of the inner integument are further advanced and the nuclei of the spore mother cells are in synapsis or late prophase stages of reduction division.

The collection of June 17 shows the funiculi of the ovules still longer, the tip of the ovule still more bent, the inner integument grown until it reaches the tip of the ovule, and most of the spore mother cells in metaphase or anaphase of reduction division. By this time the pollen tubes have reached the cavity of the ovary, having grown downward from the style along the inner surface of the ovary wall on either side of the base of each placenta. On June 21, 16 day after pollination, the ovules appear as in figure 3. The lobe of the inner integument has grown around the nucellus, forming the micropyle, and the lobe of the outer integument has begun to lengthen. The internal cells of the chalazal part of the ovule have begun to enlarge. Megasporogenesis is completed and the megagametophyte has enlarged and matured. The megagametophyte will be seen to contain seven, or possibly eight, nuclei. Pace (**9**) reports that, in *Cypripedium parviflorum*, *C. pubescens*, *C. candidum*, and *C. spectabile* (*C. hirsutum* Mill.), the megaspore mother cell divides once and the chalazal daughter cell enlarges and becomes the megagametophyte. The mature megagametophyte consists of four nuclei, two synergids and an egg at the micropylar end, and the fourth nucleus in the center or at the antipodal end. Although I have made no exhaustive study of megagametophyte development, it has been noticed that many of them in my preparations contained more than four nuclei. Rutgers (**12**), Schnarf (**15**), and Maheshwari (**7**) criticize Pace's work and list *Cypripedium* as showing a doubtful type of megagametophyte development. In view of these criticisms, I shall study the megagametophytes further and report the findings at a later time.



FIGS. 1–9.—Fig. 1, two ovules 2 days after pollination; upper one showing first divisions in formation of inner integument, lower showing spore mother cell in synapsis. Fig. 2, ovule 5 days after pollination; tip beginning to bend. Fig. 3, mature ovule 26 days after pollination, showing inner integument, micropyle, beginning of outer integument, and mature megagametophyte. Fig. 4, young seed 33 days after pollination and shortly after fertilization; 2-celled embryo, remains of megagametophyte; inner cells of chalaza and funiculus beginning to vacuolate and enlarge. Fig. 5, seed 33 days after pollination, young embryo with suspensor; funiculus and outer integument elongating. Fig. 6, chalazal portion of seed 72 days after pollination, showing chalazal cavity, remains of megagametophyte, nucellus disintegrated, inner integument shriveled, inner and side walls of cells of outer layer of funiculus and outer integument thickened, inner cells of funiculus and outer integument disintegrating, embryo enlarging. Fig. 7, central portion of seed 72 days after pollination, showing larger embryo and its relation to cells of seed coat; inner integument still more shriveled. Fig. 8, longitudinal section of entire seed; seed coat consisting of funiculus and outer integument; shriveled inner integument forming envelope around embryo; embryo suspended at apical end. Fig. 9, ripe seed; transparent coat consisting of single layer of cells; embryo suspended in pocket of air.

EMBRYO AND SEED COAT

During the season of 1936, when the material for this study was collected, fertilization occurred early in July, between 26 and 33 days after pollination. Within a week after fertilization, the zygote had divided by a wall transverse to the long axis of the megagametophyte (fig. 4). The daughter cell on the micropylar side of the embryo does not divide again, but becomes the single-celled suspensor. The other daughter cell usually divides by a wall parallel to the first (fig. 5), but sometimes by a wall perpendicular to it. Division continues in three planes, and by August 16, in ovaries pollinated on June 5, the embryo consists of an oval mass of undifferentiated meristematic cells, and a single-celled suspensor (fig. 6), which eventually disappears (fig. 7). No endosperm is produced.

While the embryo is growing, the whole ovule undergoes rapid enlargement, due primarily to the intake of water and the vacuolation of the cells. Early indications of this are seen in figure 3, where the inner cells in the chalazal region are somewhat enlarged. Further stages in this enlargement are seen in figures 4 and 5. The cells of the outer integument divide and elongate so that the outer integument grows beyond the tip of the inner integument. The funiculus also elongates and pushes the young seed out into the cavity of the capsule (figs. 5, 6). The nucellus begins to degenerate. Eventually the enlarged inner cells of the chalaza separate and disintegrate, producing the chalazal cavity (figs. 6, 8). Those cells of the chalaza in the vicinity of the apical part of the embryo are not disrupted, however, and they form a bridge suspending the embryo, with the remains of the surrounding nucellus and inner integument, in the cavity of the seed (figs. 6–8).

When the embryo has reached a many-celled stage, the nucellus has disappeared and the inner integument has begun to shrivel and degenerate. The funiculus, chalazal region, and outer integument take part in the formation of the seed coat. The internal cells of these parts gradually disintegrate and leave the external single layer of cells as the seed coat. The inner and end walls of cells of the seed coat thicken, while the outer walls remain thin. The contents of the cells of the seed coat usually disappear.

By the middle of August, ovaries contain seeds in different stages of maturity, but most of them appear as in figure 8. The embryo is still a spherical or oval mass of undifferentiated cells. It is surrounded by an envelope which consists of the shriveled and disorganized remains of the inner integument. The embryo, with its envelope, is suspended in a cavity within the seed coat by bands of living cells at its apical end. The seed coat still consists of the two outer layers of cells of the funiculus, chalaza and outer integument, but the inner layer shows evidence of disorganization.

No vascular tissue is present in the funiculus, and all food and water must therefore pass from cell to cell through the funiculus and the suspending bridge

of living cells to the embryo. The parenchymatous cells of the ovary wall and placenta are filled with starch grains throughout the summer. Starch appears early in the inner parenchymatous cells of the ovule and disappears only when they disintegrate, toward the close of the period of seed maturation. No starch is found in the embryo.

Mature seed

Capsules usually begin to dry and turn brown and seeds begin to ripen in early September, in Evanston. A mature seed is shown in figure 9. The seed coat is now a single-layered, transparent sac, open at the end which breaks from the placenta and closed at the opposite end. All the cells formerly inside this layer have disintegrated. The embryo is surrounded by the shriveled inner integument, which can be removed by dissection. The embryo, with its envelope, is suspended in the center of the sac by a few strands of cells which can be seen adhering to the embryo when it is removed from the seed coat. The embryo is bounded by an epidermal layer which is only slightly differentiated. In some embryos the cells at the apical, or bud, end are smaller and more densely filled with cytoplasm than are those at the opposite end.

Microchemical tests were made to determine the composition of walls and inclusions of cells of the mature seed. The methods used are described in Eckerson's unpublished manual of microchemistry. The thickened inner and side walls of the cells of the seed coat give a positive test for lignin with phloroglucin and hydrochloric acid, and the thin outer walls give a positive test for cellulose with iodine and sulphuric acid. The lignin and cellulose are deposited unevenly on the walls, in strips or bars. This is especially evident on those cells near the open end of the seed. None of the walls of cells of the seed coat gives a positive test for cutin with Sudan III. Netolitzky (8) states that the inner and side walls of the cells of the seed coat are cutinized. The cells of the seed coat may be empty or may contain granules which stain yellow-brown with iodine. The walls of cells which suspend the embryo and those of the embryo itself give positive tests for cellulose. The covering of the embryo gives negative tests for lignin, cellulose, and cutin, with the methods used. The cells of the embryo, especially the large cells at the posterior end, contain droplets of oil. Starch, sugar, and nitrates are absent from the mature seed coat and embryo.

The seeds of most orchids are very light and buoyant because of their small size and their saclike, air-filled coats. They are readily carried by the wind and they float on water. The loss of seeds in nature by wind or by run-off water is probably great.

Nothing is known as to how the embryo obtains water for germination. The air inside the coat is not easily removed or replaced by water—not even when suction is applied. Since the embryo is suspended from the coat in a pocket of air,

it must obtain water for germination by imbibition through the coat and along the strands of cells by which it is suspended, or from the air itself. Since the cell walls of the coat are not cutinized, such imbibition may occur. Of course, if the coat decays, water could reach the embryo, but no information on this point was obtained.

Summary

1. The anatropous ovule is formed in the usual manner from the placenta. The enlarged archesporial cell becomes the spore mother cell, which divides by meiosis.

2. The mature megagametophyte has been reported to contain four nuclei, but in my material it seems to be of the usual 8-nucleate type. More evidence is required to settle this point.

3. The zygote divides transversely to the long axis of the megagametophyte. The micropylar daughter cell becomes a one-celled suspensor and the chalazal daughter cell becomes the embryo proper. The wall dividing the chalazal daughter cell is usually parallel to the first, but may be at right angles to it. The mature embryo is a spherical, or spheroidal, mass of undifferentiated—or only slightly differentiated—cells which have cellulose walls and which contain droplets of oil, but no starch or sugar.

4. The nucellus disintegrates at an early stage of seed development. The inner integument shrivels and becomes a skinlike covering around the embryo. No endosperm is produced.

5. The seed coat is formed from the funiculus, chalaza, and outer integument, the inner cells of which disintegrate, leaving only the outer layer. The inner and side walls of the cells of the seed coat are composed of lignin and the outer walls of cellulose. No cutin was found on the seed coat.

6. A cavity is formed at the chalazal end of the seed by rupture and disintegration of inner cells and at the micropylar end by growth of the funiculus and outer integument. The embryo is suspended in this air-filled cavity by certain cells of the chalazal region.

7. The air inside the seed coat makes the seeds buoyant and may make it difficult for the embryo to obtain water for germination.

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EFFECT OF LIGHT AND TEMPERATURE ON FLORAL INITIATION IN COCKLEBUR AND BILOXI SOYBEAN[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 516

WILLIAM E. SNYDER

(WITH FIVE FIGURES)

Introduction

In recent work with Biloxi soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*), HAMNER (6) has shown that photoperiodic induction takes place as a result of reactions which occur during both the light and the dark period of a photoinductive cycle. Disregarding the results dealing with light intensity and temperature, the following scheme may illustrate the response on the part of the plant to given treatments. For purposes of simplicity, the only variations introduced are those of duration of the photoperiods and the dark periods during particular treatments with cyclic alternations of light and darkness.

1. If the light period is of a given duration (about 12 hours) and the dark period is also of a given duration, the plants will flower as a result of treatment.

2. If the dark period is shorter than 8 hours, the plants will fail to flower, regardless of the duration of the photoperiod.

3. If the photoperiod is very short, even though the dark period is relatively long, the plants may fail to flower; the response depends upon the previous conditions to which the plant has been exposed.

4. If both the photoperiods and the dark periods are relatively short, the plants will fail to flower, and such treatment serves to inhibit flowering following subsequent photoperiodic cycles which might otherwise prove effective in bringing about induction.

As a result of these observations, HAMNER has presented the following relationship with regard to Biloxi soybean and cocklebur. If A represents the changes or conditions related to exposure to light, and B represents those related to exposure to darkness, then A, B→C represents the interrelationship between A and B which brings about initiation of floral primordia, the latter condition being designated by C.

This interaction takes place in condition (1) above. In condition (2), the reac-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Part of the equipment was obtained through a grant to Dr. K. C. Hamner from the American Association for the Advancement of Science.

tions leading to floral initiation do not take place because of limitation in the effectiveness of B. Under condition (3), floral initiation does not take place because of limitation in the effectiveness of A. Under condition (4), the reactions are limited by the ineffectiveness of both A and B. In this last case exposure to very short cycles of alternating light and darkness results in decreased effectiveness of both A and B, regardless of the previous treatments; subsequent to such cycles, conditions must be attained which will result in an increase in the effectiveness of both A and B in order that floral initiation may result.

Thus the cocklebur plants growing on a long photoperiod in the greenhouse remain vegetative under condition (2). Such plants may reach a high degree of effectiveness of A, and are induced to flower by a single treatment of a long dark period, since their flowering is limited only by the effectiveness of B. If such plants are removed from the greenhouse and treated with a number of short cycles of alternating light and darkness, however, the effectiveness of A decreases. Subsequently such plants must be exposed to a photoinductive cycle, which includes both a relatively long light period and a relatively long dark period.

The present work deals with these various interrelationships and the influence of temperature, light intensity and duration, and other environmental factors upon these interactions.

General methods

Two short-day plants, cocklebur and Biloxi soybean, were employed. The experiments with cocklebur were performed during the summer and early autumn of 1939, while the experiments with soybean were conducted during the spring and summer of 1940.

The cockleburs were grown from seed collected in the vicinity of Chicago in November, 1938. The burs were stored out-of-doors until desired for use. The seeds were removed from the fruit, scratched slightly to rupture the seed coats, and planted within a few days in light potting soil. Before germination of the seeds, the flats were placed on a well-lighted bench in the greenhouse, where supplementary illumination of about 100 foot-candles at the soil surface was provided by Mazda filament light from sundown until 2:00 A.M., making a photoperiod never less than 18 hours. The conditions on this bench will subsequently be referred to as those of long photoperiod. Cocklebur and other short-day plants have been grown under these conditions for more than a year without showing evidence of floral initiation when dissected. When the seedlings were about 4 inches tall they were transplanted to $3\frac{1}{2}$-inch clay pots.

Four seeds of Biloxi soybean were planted in $4\frac{1}{2}$-inch clay pots containing garden soil and placed immediately on long photoperiod. After the seedlings had emerged and the cotyledons were well expanded, two were removed from each pot, care being taken to secure maximum uniformity of plants.

The plants were watered twice daily with tapwater and were frequently given a double-strength modified SHIVE'S (12) solution. They were exposed to conditions of long photoperiod until treated. The cockleburs were 6–8 weeks old and the soybeans 4–5 weeks old when the experiments were started. At the end of a specific treatment, if the intensity of sunlight in the greenhouse was 1000 foot-candles or more, the plants were placed immediately on long photoperiod; if less than this, the cockleburs were placed in a room at 70° F. illuminated with a carbon arc and the soybeans under white fluorescent light (1000 foot-candles or more) until the sunlight was sufficiently strong for them to be placed under conditions of long photoperiod. After treatment, all plants remained under conditions of long photoperiod for 3 weeks, at which time dissections were made. At the start of each experiment, and from time to time during them, plants which had been continuously under conditions of long photoperiod were dissected to make certain that no floral initiation had taken place. Illumination was supplied by sunlight, Mazda filament light, carbon arc light, or white fluorescent light. Intensities were determined in foot-candles by means of a standardized Weston photometer.

In experiments 1 and 2 with cocklebur, and in experiments 8–10 with soybean, the plants were treated with short cycles of alternating light and darkness, each consisting of 3 hours of darkness followed by 3 minutes of light. In the remaining experiments with cocklebur (experiments 3–7) these short cycles consisted of 2 hours of darkness followed by 2 minutes of light. The light intensity of these short photoperiods was 200 foot-candles unless otherwise indicated.

Procedure and results

Cocklebur

The experiments were of two general types: one dealt with the effectiveness of short alternating periods of darkness and light under various conditions in preventing floral initiation, and the other with the effects of light and temperature—both separate and in combination—during the photoperiod on floral initiation and development.

A. Effectiveness of short alternating periods of darkness and light under various conditions in preventing floral initiation

It has been shown that vegetative cockleburs kept constantly on long photoperiod may fail to be induced if just subsequent to a long photoperiod and prior to the 15-hour dark period a series of relatively short cycles of alternating light and dark periods are given (6). The same procedure was employed here in the first of these experiments, but in all subsequent ones it was slightly modified.

EXPERIMENT 1.—The first experiment was begun June 29, 1939. One hundred and sixty plants growing on long photoperiod were selected for uniformity. Ten of

these, designated continuous long photoperiod controls, were left on conditions of long photoperiod. Twenty were placed in a darkroom immediately; of these, ten were given a 15-hour and ten a 36-hour continuous dark period and then returned to conditions of long photoperiod. One group of ten plants was given a single short cycle of 3 hours of darkness and 3 minutes of light, subjected to a 15-hour dark period, and then returned to conditions of long photoperiod; another lot was given two of these short cycles, followed by a 15-hour dark period, and returned to long photoperiod; and so on. The maximum number of short cycles which any lot received was twelve. The remaining ten plants were given twelve of these cycles and returned immediately to long photoperiod without being exposed to a 15-hour dark period. The results (table 1) indicate that the cyclic treatment,

TABLE 1

EFFECT OF NUMBER OF SHORT CYCLES, EACH CONSISTING OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT, ON FLORAL INITIATION IN COCKLEBUR WHEN PLANTS RECEIVED A LONG DARK PERIOD IMMEDIATELY SUBSEQUENT TO TREATMENT WITH SHORT CYCLES. TEN PLANTS IN EACH LOT

No. of short cycles	0	0	0	1	2	3	4–12
Length of dark period following short cycles (hours)	0	15	36	15	15	15	15
Flowering condition after 14 days on long photoperiod	10 veg.	10 fl. pr.	10 fl. pr.	4 fl. pr., 6 veg.	10 veg.	10 veg.	10 veg.

following a long photoperiod, may prevent the effectiveness of a subsequent 15-hour dark period in initiating floral primordia.

EXPERIMENT 2.—On the basis of the results of experiment 1, a second was carried out in August, 1939. Three relatively short cycles of 3-hour dark periods and 3-minute light periods, shown by the preceding results to be sufficient to prevent initiation, were used. Upon dissection, however, it was found that many of the controls (which received a 15-hour dark period following the three cycles) had initiated floral primordia.

June 29 was intermittently cloudy, and the light intensity was therefore rather low. Experiment 1 was begun at 10:00 A.M. on that day, so these plants had been subjected to approximately 5 hours of low-intensity light. Experiment 2 was started at 5:00 P.M. following an exceptionally bright day. Since this difference might have had an important effect on the number of relatively short alternating light and dark periods required to inhibit floral initiation, a thorough investigation was made of the effects of the environmental factors on the effectiveness of the cyclic treatment.

For uniformity, all remaining experiments (3–10) were begun at 5:00 P.M. fol-

lowing at least 10 hours of sunlight of 3000–5000 foot-candles' intensity. Experiments 3–7 were carried out during the first week of September, and dissections were made during the first week of October.

EXPERIMENT 3.—Three groups of vegetative plants, each consisting of 168 plants, were exposed continuously to light for 36 hours, one group at an intensity of 50 foot-candles, another at 500, and another at 5000. From each of these groups sixteen plants were placed in the darkroom. Of each group of sixteen, half received a 12-hour and half a 36-hour continuous dark period and then returned to long photoperiod. From each of the original groups, 152 plants were given short cycles of 2 hours of darkness followed by 2 minutes of light of 200 foot-

TABLE 2

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF VARIOUS LIGHT INTENSITIES DURING 36-HOUR PHOTOPERIOD BEFORE CYCLIC TREATMENT CONSISTING OF 2 HOURS OF DARKNESS AND 2 MINUTES OF LIGHT. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

No. of short cycles	0	0	0	1	2	3	4	5–18	18
Length of dark period following short cycles (hours)	0	12	36	12	12	12	12	12	0
Intensity of 36-hour photoperiod:									
50 f.c.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
500 f.c.	8 veg.	8 fl.	8 fl.	8 fl.	5 fl., 3 veg.	8 veg.	8 veg.	8 veg.	8 veg.
5000 f.c.	8 veg.	8 fl.	8 fl.	8 fl.	8 fl.	6 fl., 2 veg.	4 fl., 4 veg.	8 veg.	8 veg.

candles at 70° F. Lots of eight plants were then removed from each group at the end of every cycle and subjected to a 12-hour continuous dark period before being returned to long photoperiod. One set of plants, however, which had received each of the light intensities prior to the cyclic treatment, was returned directly to long photoperiod after eighteen cycles, no 12-hour dark period having preceded this return. Eight plants, selected for controls for experiments 3–7 and left on long photoperiod, were vegetative at the conclusion of the experiment.

The results (table 2) indicate the reason for the variation observed between experiments 1 and 2. Apparently the degree of effectiveness of the cyclic treatment is directly related to the intensity and duration of light which the plants receive just prior to the short cycles of darkness and light; that is, plants exposed to high-light intensity preceding the cyclic treatment require more of these short cycles to prevent subsequent floral initiation than do those exposed to a relatively low-light intensity prior to the cyclic treatment.

Since these short cycles were effective in inhibiting subsequent floral initiation in the treatments, the effect of continuous photoperiod of low light intensity, as compared with these short cycles, was next determined. An attempt was made also to determine the effect of variation in the light intensity during the short photoperiods of these short cycles.

EXPERIMENT 4.—Four hundred and eighty-eight cocklebur plants, growing on long photoperiod, were selected for uniformity. Sixteen of these were placed in the darkroom, eight being returned to long photoperiod after a 12-hour and

TABLE 3

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF CYCLIC TREATMENT CONSISTING OF 2 HOURS OF DARKNESS AND 2 MINUTES OF LIGHT OF VARIOUS INTENSITIES AND OF CONTINUOUS LIGHT OF LOW INTENSITY. EACH LOT OF EIGHT PLANTS

No. of cycles	1	2	3	4	5	6	7	8	9–18	18
No. of hours of continuous darkness following cyclic treatment	12	12	12	12	12	12	12	12	12	0
Flowering condition after 21 days on long photoperiod, when cycle consisted of:										
2 hours of darkness plus 2 minutes of light at:										
2000 f.c.	8 fl.	8 fl.	5 veg., 3 fl.	4 veg., 4 fl.	7 veg., 1 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
200 f.c.	8 fl.	8 fl.	2 veg., 6 fl.	4 veg., 4 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
20 f.c.	8 fl.	8 fl.	3 veg., 5 fl.	5 veg., 3 fl.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.	8 veg.
2 hours of continuous light at 20 f.c.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	8 fl.	7 veg., 1 fl.	8 veg.	8 veg.

eight after a 36-hour continuous dark period. These control plants initiated primordia. One hundred and twelve plants were placed under continuous light of 20 foot-candles. Every 2 hours for the first 20 and every 4 hours for the next 16, lots of eight plants each were removed to the darkroom for 12 hours and then immediately returned to long photoperiod. Three lots of 120 plants each were given short cycles with the 2-minute light periods at intensities of 20, 200, and 2000 foot-candles, respectively. Each of the eighteen short cycles consisted of 2 hours of darkness followed by 2 minutes of light. Lots of eight plants were removed from each intensity at the end of every cycle, given a 12-hour continuous dark period, and returned to long photoperiod. At the completion of eighteen cycles an additional group of eight plants from each intensity was returned directly to long photoperiod with no intervening dark period.

The results (table 3) show that for cocklebur, cycles with brief periods of light of all three intensities are effective in inhibiting floral initiation, even though the plants are given a subsequent 12-hour dark period. Those plants given cycles with 2-minute light periods at 2000 foot-candles required seven cycles for such inhibition, while those given 20 or 200 foot-candles required only five cycles. The plants given 18 hours or more of continuous light of low intensity (20 foot-candles) also failed to initiate floral primordia, even when subsequently given a 12-hour dark period.

Table 3 indicates that light intensity during the treatment of short cycles may influence the results. In the following experiment an attempt was made to determine what effect temperature might have on treatment with short cycles.

TABLE 4

EFFECT ON FLORAL INITIATION IN COCKLEBUR OF VARIOUS TEMPERATURES DURING SHORT CYCLES CONSISTING OF 2-HOUR DARK AND 2-MINUTE LIGHT PERIODS. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

No. of short cycles	1	2	3	4	5	6–18	18
Length of dark period following short cycles (hours)	12	12	12	12	12	12	0
Flowering condition after 21 days on long photoperiod, following short cycles at:							
40° F.	8 fl.	8 fl.	1 veg., 7 fl.	2 veg., 6 fl.	7 veg., 1 fl.	8 veg.	8 veg.
75° F.	8 fl.	8 fl.	2 veg., 6 fl.	4 veg., 4 fl.	8 veg.	8 veg.	8 veg.
95° F.	8 fl.	8 fl.	1 veg., 7 fl.	3 veg., 5 fl.	8 veg.	8 veg.	8 veg.

EXPERIMENT 5.—The continuous long photoperiod controls of this experiment are those of experiment 3, and the 12- and 36-hour continuous dark-period controls (not given the short-cycle treatment) are those of experiment 4. Three hundred and sixty plants were divided into three lots of 120 plants each and subjected to the short-cycle treatment at 40°, 75°, and 95° F., respectively. Lots of eight plants were removed from each group at the end of every short cycle, given a 12-hour continuous dark period at 70° F., and returned to long photoperiod. The results of this experiment are given in table 4. The differences in response to the three temperature groups are insignificant and indicated that the temperatures used were without influence on the effectiveness of the alternating dark and light periods in preventing floral initiation.

B. DURATION AND INTENSITY OF LIGHT AND TEMPERATURE REQUIREMENTS OF PHOTOPERIOD PRECEDING DARK PERIOD

Since it has been observed that floral initiation could be prevented by treating plants with short cycles of alternating darkness and light before subjecting them

to a continuous dark period of 9 hours or more, it was decided to test the conditions under which plants might resume a condition permitting initiation by giving some which had received eighteen short cycles a controlled photoperiod before subjecting them to the continuous dark period of at least 12 hours.

EXPERIMENT 6.—Eighty vegetative cocklebur plants were selected from long photoperiod and given eighteen cycles of alternating 2-hour dark periods and 2-minute light periods (200 foot-candles) at 75° F. Ten plants were returned to long photoperiod immediately after the eighteen cycles, and served as checks. The remaining seventy were divided equally into seven groups, each of which was given one of the following treatments:

2 hours of 2000 f.c. carbon arc light +22 hours darkness
4 hours of 2000 f.c. carbon arc light +20 hours darkness
6 hours of 2000 f.c. carbon arc light +18 hours darkness
8 hours of 2000 f.c. carbon arc light +16 hours darkness
10 hours of 2000 f.c. carbon arc light +14 hours darkness
10 hours of 100 f.c. Mazda filament light +14 hours darkness
10 hours of 10 f.c. Mazda filament light +14 hours darkness

Each cycle was repeated three times, at the conclusion of which the plants were returned immediately to long photoperiod, where they remained for 21 days.

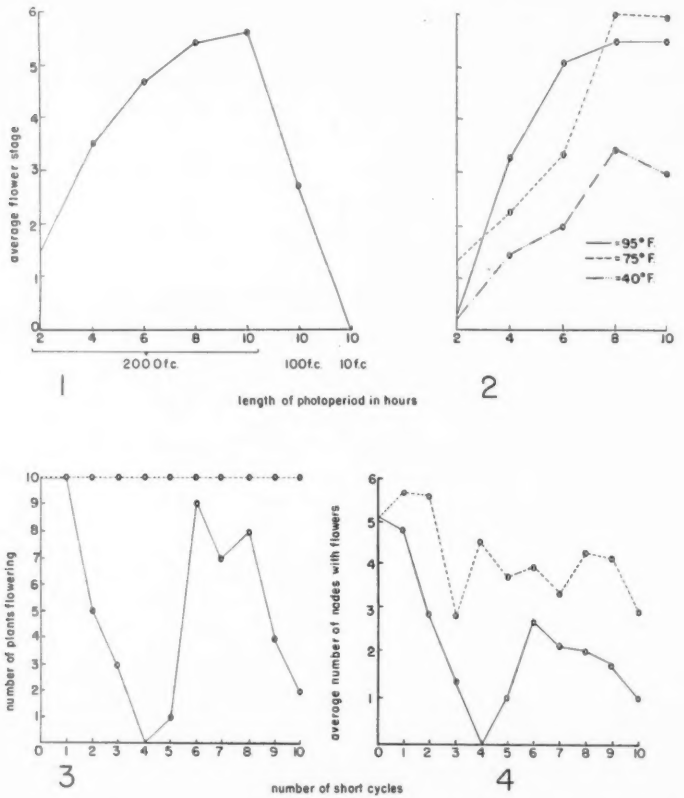
TABLE 5

FLOWERING RESPONSE OF COCKLEBUR TO VARIOUS PHOTOINDUCTIVE CYCLES GIVEN AFTER 18 CYCLES OF ALTERNATING 2-HOUR DARK AND 2-MINUTE LIGHT PERIODS. EACH PHOTOINDUCTIVE CYCLE REPEATED THREE TIMES. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF TEN PLANTS

PHOTOINDUCTIVE TREATMENT	NO. OF PLANTS	
	VEG.	FL. PR.
2 hours of 2000 f.c.+22 hours of darkness	2	8
4 hours of 2000 f.c.+20 hours of darkness	0	10
6 hours of 2000 f.c.+18 hours of darkness	0	10
8 hours of 2000 f.c.+16 hours of darkness	0	10
10 hours of 2000 f.c.+14 hours of darkness	0	10
10 hours of 100 f.c.+14 hours of darkness	0	10
10 hours of 10 f.c.+14 hours of darkness	10	0
Controls (continuously on long day)	10	0

In two groups, the controls and those given three cycles of 10 foot-candles Mazda filament light for 10 hours, there was no initiation of floral primordia (table 5). Two of the plants exposed to three cycles of 2000 foot-candles of carbon arc light for 2 hours and 22 hours of darkness also failed to initiate primordia. The apical meristems had become flower primordia in all other plants. The stages of

flower development were arbitrarily classified, and an estimate as to the effectiveness of the different treatments was obtained. Stage 1 represents the first indica-



FIGS. 1–4.—Fig. 1, following eighteen cycles of alternating short dark and light periods, effect of light intensity and duration of photoperiod of 24-hour photoinductive cycle on floral development in cocklebur. Each cycle repeated three times. Data taken 21 days after completion of treatment. Fig. 2, effect on floral development of temperature during photoperiods of various durations, following eighteen cycles of alternating short dark and light periods. Each photoperiod 2000 foot-candles, followed by 14-hour dark period at 70° F. Each cycle repeated three times. Data taken 21 days after completion of treatment. Figs. 3, 4, effect on initiation in Biloxi soybean of ten photoinductive cycles which include short cycles consisting of 3 hours of darkness and 3 minutes of light (200 f.c.). Short cycles preceding dark period represented by broken line; those following, by solid line. Data taken 21 days after completion of treatment.

tion of initiation; stage 6, macroscopic flowers; the other stages range between. Figure 1 represents the average stage of flowering of all plants in a group and shows

that the longer the duration and the stronger the intensity of the photoperiod, the more rapid the subsequent rate of floral development.

EXPERIMENT 7.—One group of workers (**4, 5, 10, 11, 13, 14**) has reported that variations in temperature during the photoperiod may alter the length of the light period required for floral initiation in many plants. Others (**4, 5, 7, 9, 10**) have shown that the length or number of critical dark periods may be adversely affected by temperature conditions during the dark period.

Experiment 7 shows that the temperature conditions prevailing during the photoperiod affect initiation and subsequent flower development. It varies from ex-

TABLE 6

FLOWERING RESPONSE OF COCKLEBUR TO VARIATIONS IN TEMPERATURE DURING PHOTOPERIOD OF VARIOUS PHOTOINDUCTIVE CYCLES FOLLOWING 18 CYCLES OF ALTERNATING 2-HOUR DARK AND 2-MINUTE LIGHT PERIODS. TEMPERATURE OF DARK PORTION OF PHOTOINDUCTIVE CYCLE 70° F. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF EIGHT PLANTS

	Temperature during photoperiod					
	40° F.		75° F.		95° F.	
	Veg.	Fl.	Veg.	Fl.	Veg.	Fl.
After 18 cycles of alternating light and dark periods, subjected to three photoinductive cycles:						
2 hours of 2000 f.c.+14 hours dark	7	1	1	7	7	1
4 hours of 2000 f.c.+14 hours dark	1	7	0	8	0	8
6–10 hours of 2000 f.c.+14 hours dark	0	8	0	8	0	8
Controls (continuously on long photoperiod)	8 vegetative, 0 flowering					
After 18 cycles transferred directly to long photoperiod	8 vegetative, 0 flowering					
After 18 cycles transferred to 12-hour dark period, then to long photoperiod	8 vegetative, 0 flowering					
Transferred to 14-hour dark period immediately after photoperiod, then to long photoperiod	0 vegetative, 8 flowering					

periment 6 in two respects: (*a*) all plants were given 12-hour dark periods, and (*b*) three temperature conditions prevailed during the photoperiod. The results (table 6) show that initiation was inhibited at low and high temperatures, but only when the treatment consisted of three cycles of 2 hours of 2000 foot-candles of carbon arc light and 12-hour dark periods. Significant variations were found in the rate of development of the flower primordia (fig. 2). In general, at the higher temperatures there was greater increase in the rate of flower-bud development than at the low temperature. At all three temperatures a marked increase in the rate of flower-bud development followed three photoinductive cycles whose photoperiods

were 8 hours long. Plants given three cycles with 10-hour photoperiods had not developed so far as those given an 8-hour photoperiod.

Biloxi soybean

Relatively short alternating dark and light periods were inserted into the photoinductive cycle of another short-day plant to test the effectiveness of the cyclic treatment in preventing floral initiation. Soybean was selected because of the data available relative to its photoperiodic relationships. As in the case of cocklebur, short cycles were given between the photoperiod and the dark period of each photoinductive cycle, and similar short cycles were inserted between each photoinductive cycle (following the dark period of each photoinductive cycle).

EXPERIMENT 8.—On May 3, 1940, 250 soybeans which had been on long photoperiods for 5 weeks were selected. Five lots of ten plants each were used as controls and given one of the following treatments:

1. Left continuously on long photoperiod.
2. Given 240 hours of white fluorescent light of 600 foot-candles, then returned to long photoperiod.
3. Given 240 hours of continuous darkness, then returned to long photoperiod.
4. Given 80 short cycles (240 hours) consisting of 3 hours of darkness and 3 minutes of light (200 foot-candles Mazda filament light), then returned to long photoperiod.
5. Given ten photoinductive cycles consisting of 10 hours of light followed by 14 hours of darkness, then returned to long photoperiod.

All plants of lots 1–4 were vegetative, but those of lot 5 were flowering at the conclusion of the experiment. The remaining 200 plants were divided into two equal lots, and at 5:00 P.M., following a photoperiod of more than 10 hours of sunlight of 3000–5000 foot-candles, one (group A) was placed in the darkroom, while the other (group B) was placed in a darkroom equipped to give frequent short periods of Mazda filament light.

Following a 14-hour continuous dark period, the 100 plants of group A were removed to the short-cycle room. One lot of ten plants received one short cycle consisting of 3 hours of darkness followed by 3 minutes of light, then placed under white fluorescent light (600 foot-candles) for 10 hours, and finally returned to the darkroom for 14 hours. Another lot received two of these short cycles following the 14-hour dark period; a third received three of these cycles, etc. Each of these treatments (10 hours of light plus 14 hours of darkness plus 1–10 short cycles) was repeated ten times. At the conclusion of a given treatment the plants were returned to conditions of long photoperiod in the greenhouse—provided the intensity of the sunlight exceeded 1000 foot-candles—or were placed under white fluorescent light until such time as the intensity of the sunlight was 1000 foot-candles or more.

Further, any group which completed the treatment during the late afternoon was kept under the fluorescent light until the following morning.

Of the 100 plants of group B, placed in the short-cycle room at the start of the experiment, one lot of ten plants remained for one short cycle, another for two, and so on. Upon removal from this room, the plants were given a 14-hour continuous dark period, followed by 10 hours of white fluorescent light, and then returned to the short-cycle room for the specified number of short cycles. Each of these treatments was likewise repeated ten times, and at the conclusion of a given treatment the plants were returned to conditions of long photoperiod.

The results (fig. 3) are striking, in that short cycles inserted before the dark period were ineffective in preventing floral initiation, while the inhibitory effect of short cycles following the dark period appears rhythmical. Although all plants were flowering when the short cycles preceded the dark period, some inhibitory effect on flowering is evidenced by the average number of nodes with flowers per plant. Figure 4 shows that the same general trends of inhibition of the number of nodes with flowers is affected by short cycles inserted either before or after the dark period, as well as by the effect of short cycles inserted after the dark period on the total number of plants flowering. A comparison of the average number of nodes per plant with flower primordia of comparable treatments from groups A and B shows that in no instance was there a greater number of nodes with primordia when the short cycles followed the dark period than when they preceded it. Evidently short cycles inserted after the dark period of the photoinductive cycle are much more effective in suppressing the flowering response.

EXPERIMENT 9.—This experiment was performed at the same time as experiment 8, hence the controls for both were the same. Seven hundred soybeans which had been on continuous long photoperiods were selected for uniformity and divided into seven groups of 100 plants each. These groups were subjected to cycles as follows:

1. 10 hours of light, then 14 hours of continuous darkness.
2. 10 hours of light followed by one short cycle (3 hours of darkness and 3 minutes of 200 foot-candles of Mazda filament light), then 14 hours of continuous darkness.
3. 10 hours of light followed by five short cycles, then 14 hours of continuous darkness.
4. 10 hours of light followed by ten short cycles, then 14 hours of continuous darkness.
5. 10 hours of light followed by 14 hours of continuous darkness, then one short cycle.
6. 10 hours of light followed by 14 hours of continuous darkness, then five short cycles.

7. 10 hours of light followed by 14 hours of continuous darkness, then ten short cycles.

Ten plants from each group were removed to conditions of long photoperiod at the end of each cycle, ten cycles being required to remove all plants from any group.

The effect of the short-cycle treatments, combined with a varying number of photoinductive cycles, upon the total number of plants flowering per treatment is given in table 7. In no instance were flowers induced when less than three photoinductive cycles were used; and as has been previously reported (**1, 3, 9**), maximum flowering occurred when a relatively large number of photoinductive cycles were given.

TABLE 7

EFFECT ON FLORAL INITIATION IN SOYBEAN OF VARYING NUMBER OF PHOTOINDUCTIVE CYCLES WHICH INCLUDE SHORT CYCLES CONSISTING OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT (200 F.C.). DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF TEN PLANTS

TREATMENT	NO. OF PLANTS FLOWERING AFTER NUMBER OF PHOTOINDUCTIVE CYCLES AS INDICATED									
	1	2	3	4	5	6	7	8	9	10
No. of short cycles inserted in photoinductive cycle before 14-hour dark period:										
1	0	0	2	2	8	8	9	10	10	10
5	0	0	0	0	0	3	0	3	8*	9*
10	0	0	0	0	0	2	1	6	8	10
No. of short cycles inserted in photoinductive cycle after 14-hour dark period:										
1	0	0	0	1	4	9	10	10	10	10
5	0	0	0	0	0	0	0	0	2	1
10	0	0	0	0	2	0	1	5	3	2
No short cycles included in photoinductive cycle	0	0	6	8	10	10	10	10	10	10
Continuous long photoperiod	0 flowering; 10 vegetative									

* One dead plant in this lot.

One short cycle inserted either before or after the dark period of the photoinductive cycle had some effect when relatively few photoinductive cycles were given (seven or less), but when eight or more comprised the treatment all plants showed some degree of flowering. If five short cycles were so inserted before or after one to eight photoinductive cycles, floral expression was much more inhibited. Five short cycles inserted before the dark period of nine or ten photoinductive cycles did not materially inhibit initiation, but when inserted after the dark period, very few plants were flowering. In general, when ten short cycles were included the floral response ranged between these two.

EXPERIMENT 10.—This was designed to test the results obtained in experiment 8 as well as to compare the effectiveness of continuous light of low intensity with short cycles which included 3-minute light periods of low and high intensity in preventing floral initiation in soybean.

The plants were 30 days old at the start of the various treatments and had been under long photoperiod in the greenhouse for that entire time. The same precautions employed before and after treatments in other experiments were used here. Table 8 gives the relative humidity and temperature readings made every 3 hours, and maximum, minimum, and averages for the five rooms used. Fluctuations in both temperature and relative humidity were gradual.

White fluorescent light (1200 foot-candles) was used for the 10-hour photoperiods, and similar light of 20 foot-candles was used for the low intensity con-

TABLE 8

Room	Temperature (° F.)			Percentage relative humidity		
	Max.	Min.	Ave.	Max.	Min.	Ave.
Darkroom	86	76	82	80	63	77
Continuous light (20 f.c.)	88	79	84	80	47	75
Continuous light (1200 f.c.)	89	79	85	85	53	75
Short cycle (200 f.c.)	84	78	81	84	64	75
Short cycle (20 f.c.)	83	76	80	87	64	75

tinuous light treatment. The 3-minute light periods of the cyclic treatment were of 20 and 200 foot-candles Mazda filament light.

On July 8, 1940, 800 soybeans were selected for uniformity. Twenty of these were dissected immediately and every node was found to be vegetative. Eighty more were designated controls and divided into four lots of twenty plants, each receiving one of the following treatments:

1. Continuous long photoperiod. All plants vegetative.
2. Ten photoinductive cycles consisting of 10-hour photoperiods and 14-hour dark periods, then returned to long photoperiod. All plants flowering at conclusion of experiment. Average number of nodes with flowers, 5.3.
3. Ten days of continuous light of 20 foot-candles, then returned to long photoperiod. All plants vegetative at conclusion of experiment.
4. Ten days of continuous light of 1200 foot-candles, then returned to long photoperiod. All plants vegetative at conclusion of experiment.

Two groups of 200 plants each were given ten cycles consisting of 10 hours of light, followed by 14 hours of continuous darkness, followed by a given number of short cycles of alternating 3-hour dark periods and 3-minute light periods. One group received a high-intensity light (200 foot-candles) during the 3-minute period,

while the other group received a low-intensity light (20 foot-candles) during this period. One lot of twenty plants from each group received 1, 4, 6, 8, 10, 11, 12, 13, 14, and 15 short cycles. The remaining plants were divided into fifteen lots of twenty plants, each lot receiving ten cycles of 10 hours of bright light (1200 foot-candles) plus 14 hours of continuous darkness plus a given number of hours of continuous light at 20 foot-candles. One lot received 3 hours of the low-intensity light

TABLE 9

EFFECT ON FLORAL INITIATION IN SOYBEAN OF SHORT CYCLES OF 3 HOURS OF DARKNESS AND 3 MINUTES OF LIGHT OF VARIOUS INTENSITIES OR CONTINUOUS LIGHT OF LOW INTENSITY FOLLOWING EACH DARK PERIOD OF TEN PHOTOINDUCTIVE CYCLES. DATA TAKEN 21 DAYS AFTER COMPLETION OF TREATMENT. EACH LOT OF TWENTY PLANTS

No. of short cycles following each dark period of photoinductive cycle	Intensity of light of short cycles								Continuous light (20 f.c.)*			
	20 f.c.				200 f.c.							
	No. of plants			Ave. no. nodes with fl.	No. of plants			Ave. no. nodes with fl.	No. of plants			Ave. no. nodes with fl.
	Fl.	Veg.	Dead		Fl.	Veg.	Dead		Fl.	Veg.	Dead	
1	20	0	0	4.2	19	1	0	4.1	20	0	0	3.5
2									2	17	1	2.5
3									0	20	0	0.0
4	5	14	1	2.0	0	20	0	0.0	0	19	1	0.0
5									1	19	0	2.0
6	6	11	3	2.5	4	14	2	1.8	9	10	1	1.7
7									5	13	2	2.0
8	9	4	7	4.0	15	2	3	3.5	3	12	5	2.7
9									0	14	6	0.0
10	1	6	13	2.0	1	11	8	2.0	0	13	7	0.0
11	0	5	15	0.0	1	9	10	2.0	0	11	9	0.0
12	0	3	17	0.0	0	0	20	0.0	0	6	14	0.0
13	0	2	18	0.0	1	3	16	3.0	0	4	16	0.0
14	3	3	14	4.0	0	2	18	0.0	0	12	8	0.0
15	1	2	17	3.0	0	0	20	0.0	0	6	14	0.0

* Each short cycle represents 3 hours of continuous light.

following the dark period, a second received 6 hours, a third 9 hours, etc., to a maximum of 45 hours.

A number of the plants of some treatments did not survive the experimental period. Apparently this was due to the relatively short length and low intensity of the 10-hour photoperiods which made up each photoinductive cycle. The results of experiment 10 are shown in table 9.

The results of experiment 10 correspond in general to those obtained in experiment 8 (figs. 3, 5). Relatively short alternating periods of light and darkness, as well as continuous light of low intensity, when inserted after the 14-hour dark

period of the photoinductive cycle may reduce the photoinductive effect of such a cycle. The effectiveness of such treatment appears rhythmical (fig. 5). There is a rapid decrease in the number of plants flowering when 2–4 short cycles were so in-

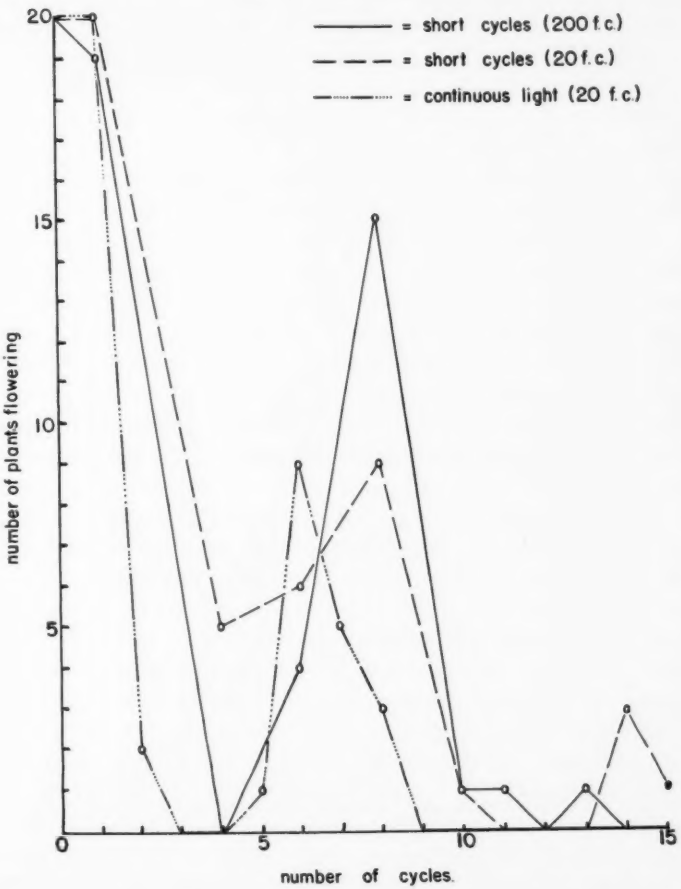


FIG. 5.—Effect on initiation in Biloxi soybean of ten photoinductive cycles which include short cycles (3 hours of darkness and 3 minutes of light or 3 hours of continuous light of low intensity) inserted after 14-hour dark period. Each short cycle represents lapse of 3 hours. Data taken 21 days after completion of treatment.

serted, then a rapid increase in the number flowering (5–9 short cycles), which is followed by another decrease. An insufficient number of the plants receiving 10–15 short cycles survived to warrant a definite statement as to their effect;

however, there is indication that an increase in the number flowering would have occurred. The average number of nodes per plant with flowers and the total number flowering of the various treatments roughly follows the same type curve (figs. 3–5). Whenever short cycles were introduced into the photoinductive cycle, regardless of their number or their place of insertion, fewer nodes per plant had flowers than occurred on plants subjected to a corresponding number of photoinductive cycles but without the short cycles.

Discussion

These results substantiate previous findings (**1, 3, 6, 7, 9**) that in both cocklebur and Biloxi soybean photoperiodic induction takes place as a result of interaction between conditions brought about during exposure both to light and to darkness. This cyclic alternation of light and dark periods, when of appropriate duration, intensity, and frequency, results in photoperiodic induction. Under certain of these conditions cocklebur requires one such photoinductive cycle, and Biloxi soybean three, for photoperiodic induction. The necessary duration of the light and the dark period is dependent upon a number of factors, such as intensity and quality of light, temperature, age of plants, age and position of leaves, and so on (**1–10**). At present the nature of the interactions which result in photoperiodic induction are unknown.

The results of the present work indicate that cocklebur, when exposed to relatively bright light, may undergo certain changes or reactions which so alter the plant that if it is immediately exposed to a relatively long dark period it becomes photoperiodically induced and may subsequently initiate floral primordia. If a plant which has been exposed to such a period of bright light is exposed to relatively short alternating periods of light and darkness, however, the effectiveness of the photoperiod disappears, and even though subsequently subjected to a long dark period, initiation does not occur. The same results brought about by these relatively short alternating light and dark periods may also be produced by exposing a plant to continuous light of very low intensity, although in the latter instance the time required for the apparent lowering of the effectiveness of the previous photoperiod is much longer.

During photoperiods of bright light the establishment of A may occur at a rapid rate and there may result a higher degree of its effectiveness at the end of the photoperiod than at the beginning. Similarly, during photoperiods with low-intensity light the rate of establishment of A may be lower, and in complete darkness or during photoperiods with a very low light intensity its rate of establishment may actually be lower than the rate of its destruction, resulting in a lower level of effectiveness at the end of the period than at the beginning. If such were the case, it would be expected that a low temperature during treatment with

short cycles would result in a lower rate of decrease of the conditions brought about during the photoperiod. Such does not seem to be the case, since variations in temperature produced only slight differences in the number of short alternating cycles of light and dark periods necessary to prevent initiation.

Whatever the reasons, it seems apparent that conditions prevailing during the photoperiod influence the relative effectiveness of A. This is indicated by the preceding results, which show that a greater number of short cycles must be used to prevent photoperiodic induction following a period of bright light than following a period of relatively low light intensity; and that following a series of eighteen alternating cycles of short light and dark periods the length and intensity of photoperiods determine the subsequent return to a condition resulting in photoperiodic induction. It would appear also that the number of short cycles needed to prevent photoperiodic induction might serve as a measure of the accumulated effectiveness of A during previous exposure to light. Utilization of this method may give a quantitative basis for the measurement of the rate and degree of reaction on the part of the plant to exposure to photoperiods at various light intensities, temperatures, etc. Such a procedure has been carried out, and the results indicate that whatever the conditions brought about during exposure to the photoperiod, they take place to a greater degree during photoperiods of high light intensity than during those of lower intensity. A low temperature during the photoperiod apparently results in a slower rate of establishment of the effectiveness of A. These results are in general agreement with the response of Biloxi soybean to experiments of a similar nature and indicate the similarity in response between the two plants.

These results with cocklebur and Biloxi soybean may lead to a possible interpretation of apparently conflicting results obtained by other workers. It has been noted from time to time that photoperiodically sensitive plants may respond differently, depending upon the time of day that they receive supplementary illumination. Thus it has been the general practice, when it was desirable to expose plants to long photoperiodic conditions, to expose them to natural daylight and supplement this by continued exposure to light from Mazda lamps into the night. Contrary to this general practice, the supplementary illumination has occasionally been given from a designated time during the night until sunrise. In one case supplementary illumination followed the photoperiod of high light intensity and in the other case preceded it. A possible explanation of the conflicting results may be that with certain plants the photoperiodic induction more readily results if a period of bright illumination immediately precedes exposure to darkness. In this connection, the experiments with Biloxi soybean are of interest. While the short cycles were partially effective in inhibiting initiation, whether they immediately preceded or immediately followed the dark period, there was considerable difference in their relative effectiveness—not only with reference to the number of

plants flowering but also with reference to the number of nodes per plant which possessed floral primordia. More evidence is needed before definite conclusions can be reached, but preliminary results indicate that treatment immediately following the dark period would prove most effective.

The indications which were obtained as to the cyclic changes in sensitivity on the part of the soybean are difficult to interpret, but it would seem that utilization of cycles other than those based on 24 hours may bring about ambiguous results because of cyclic changes in sensitivity on the part of the plant which seem to follow a 24-hour cycle. It may be that future work should involve treatment of the plants from the time they are planted until the end of the experiment to avoid such conditions.

The results of experiments with both cocklebur and Biloxi soybean at least indicate that a practical method of maintaining short-day plants in the vegetative condition would be to expose them to brief periods of illumination during the night. This should prove even more effective in maintaining the plants in a vegetative condition than the common practice of exposing them to continuous illumination of low light intensity during a part of the night.

Summary

1. In cocklebur, the conditions brought about during the photoperiod may be diminished to such a point that plants fail to initiate floral primordia when subsequently subjected to a 12-hour dark period. The methods employed are the insertion between the photoperiod and the dark period of the photoinductive cycle of a given number of short alternating periods of darkness and light or of a given number of hours of continuous light of low intensity. When Biloxi soybean was treated in the same manner there was no reduction in the number of plants flowering, although there was a decrease in the number of nodes with flowers.

2. In Biloxi soybean, short cycles of light and darkness, or continuous light of low intensity, when inserted following the dark period of each photoinductive cycle, reduced considerably the number of plants flowering as well as the average number of nodes per plant with flowers. This response appears rhythmical, and there are indications that utilization of cycles other than those under which the plant has been growing may bring about ambiguous results.

3. Effective short cycles consisted of either 3 hours of darkness and 3 minutes of light or 2 hours of darkness and 2 minutes of light.

4. The use of short alternating periods of darkness and light may be a more economical method of maintaining "short-day" plants in a vegetative condition than the common practice of exposing them to continuous supplementary illumination of low intensity.

5. The intensity of the light during these short periods apparently affects the response; and in cocklebur the temperature prevailing during the cyclic treatment had little, if any, effect.

6. A range of conditions imposed during the photoperiod of a photoinductive cycle can be directly correlated with the extent or degree of floral initiation shown by cocklebur subjected to the various treatments.

7. Following eighteen short cycles, cocklebur may regain the capacity of being induced and develop flowers if subjected to three photoinductive cycles consisting of photoperiods of 2 or more hours at 2000 foot-candles or 10 hours of 100 foot-candles and 14 hours of continuous darkness. Moderate to high temperatures during the photoperiod and an increase in the intensity and duration of the light favor establishment of the conditions brought about by the photoperiod as measured in these experiments.

This research was carried out under the guidance of Dr. K. C. HAMNER. The writer is grateful to him; and also to JOHN SKOK and AUBREY NAYLOR for their assistance with the experimental work.

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EFFECT OF GROWTH SUBSTANCES ON THE ABSCISS LAYER IN LEAVES OF COLEUS[1]

R. MAURICE MYERS

(WITH NINE FIGURES)

Introduction

LAIBACH (**11**) reported that orchid pollinia inserted in the split end of petioles of debladed leaves of coleus and certain other plants delayed abscission of the petioles 11–20 days longer than that of untreated petioles. LA RUE (**12**) found that indoleacetic acid (heteroauxin), pollen, and urine delayed petiole fall for a few days when they were applied to debladed petioles of coleus leaves. A study of the effect of growth substances in relation to other factors known to affect abscission seemed desirable.

The earlier literature on abscission is extensive and will not be discussed here. It is reviewed by KENDALL (**9**), LLOYD (**13, 14, 15**), and SAMPSON (**18**). It is generally agreed that abscission is usually preceded by the development of an absciss layer followed by the disintegration of certain lamellae in the walls of cells in this layer. SAMPSON found that an absciss layer started to form in the third pair of leaves below the terminal bud of coleus plants bearing eight pairs of leaves, and that the layers were not mature until these leaves were the sixth pair below the terminal bud. He found that at the time of abscission the lamellae of the primary wall adjacent to the middle lamellae disintegrated before the middle lamellae. The cellulose properties of these lamellae disappeared and a test for pectic compounds became more intense. These facts led him to describe abscission as a change of cellulose to pectose and later to pectic acid which resulted in an excess of pectic acid over the amount of calcium necessary to maintain the solidity of the middle lamellae of the cell wall in the absciss layer. His inferences as to the nature of the changes in cellulose, however, were made in the absence of adequate chemical data. Even today the changes that may occur in these compounds in cells under the action of enzymes are not well enough known to admit a more detailed description.

Material and methods

Several thousand plants of *Coleus blumei* Benth (var. Golden Bedder) were propagated from cuttings in a greenhouse during the winter and spring. Since coleus has opposite leaves, certain treatments can be performed on one leaf while

[1] A fuller account of this research is available, see **17**.

the other of the same age is left as a control. At any particular time the plants usually have eight pairs of leaves, not counting those folded together in the terminal bud. Eight to ten plants were used for each experiment, and each experiment was repeated several times.

A number of different treatments were used and these are described separately. The growth substances used were carefully weighed and mixed with the quantity of anhydrous lanolin necessary to make the desired concentrations. The mixtures were heated until the lanolin melted and the substances were completely dispersed. Fresh mixtures were made at frequent intervals, although it was found that the growth substances remained active in the lanolin for several years. The amount of paste applied was not measured, but about the same amount was applied to each part of the plant. Controls were treated similarly with anhydrous lanolin, and all cut surfaces were coated with lanolin to prevent desiccation.

Data for the pre-abscission period were collected daily. The pots containing the plants were tapped gently at that time, as petioles in which abscission was practically complete often remained attached to the stem for several days by a small piece of dried tissue.

The internal changes were followed by the use of freehand and rotary microtome sections. Freehand sections were frequently employed because the changes taking place during abscission could be better observed. Longitudinal sections cut through the stem and petioles at each node gave comparable views of the absciss layers of the pairs of opposed leaves.

Investigation

Growth of leaves

In order to discover any possible relationship between development of the leaves, growth of the absciss layer, and subsequent abscission, the relative rates of development of the different parts of a leaf were measured.

All leaves on several plants were marked off into 4-mm. squares with a special rubber stamp. As a check, leaves of other plants were marked off in 4-mm. segments with a small brush and India ink. Microscopic examination of the cells of the petioles, especially those close to the absciss layer, were also made.

The growth of a coleus leaf (fig. 1) is similar to that of tobacco as described by Avery (1), but it is more basipetal. There is an increasing gradient of expansion from the tip of the leaf to the base of the blade. A similar gradient of expansion occurs in the midrib and petiole, almost to its base. By the time a leaf has attained one-fifth its final size the tip has practically ceased expanding. The greatest expansion is in the second and third pairs of leaves below the terminal bud; considerably less in the fourth pair; and in the fifth, expansion of the blade has ceased but the petioles of these leaves and the sixth and seventh pairs of leaves continue to

elongate. The base of the petioles distal to the absciss layer has the longest period of expansion of the entire leaf, although it does not elongate as much as the segments of the petiole immediately above it. The base of the blade has the greatest amount of expansion, which is attained before elongation of the petiole has ceased.

Growth of the absciss layer begins in the partially enlarged and vacuolated cells in the base of the petiole of the third pair of leaves below the terminal bud and continues until these leaves have become the sixth pair. The blade is 85–90 per cent expanded before the absciss layer begins to develop. There is considerable cell enlargement in the petiole distal to the absciss layer. The cells become larger than those of the stem cortex at the base of the petiole. The epidermal cells distal to the absciss layer become greatly elongated and do not increase much in width. The base of the petiole proximal to the absciss layer and a part of the stem cortex contributes little to the increase in length of the petiole.

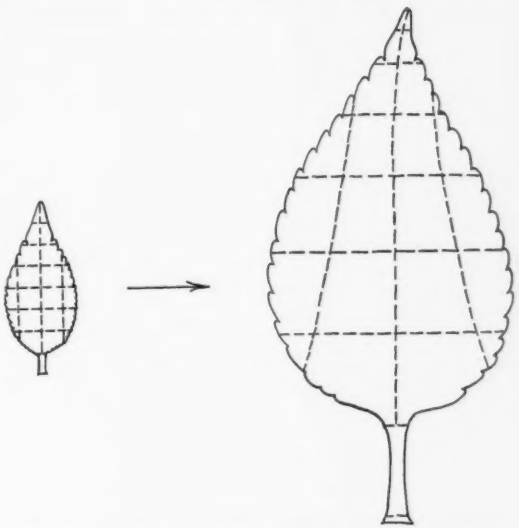


FIG. 1.—Place of expansion in growth of typical coleus leaf

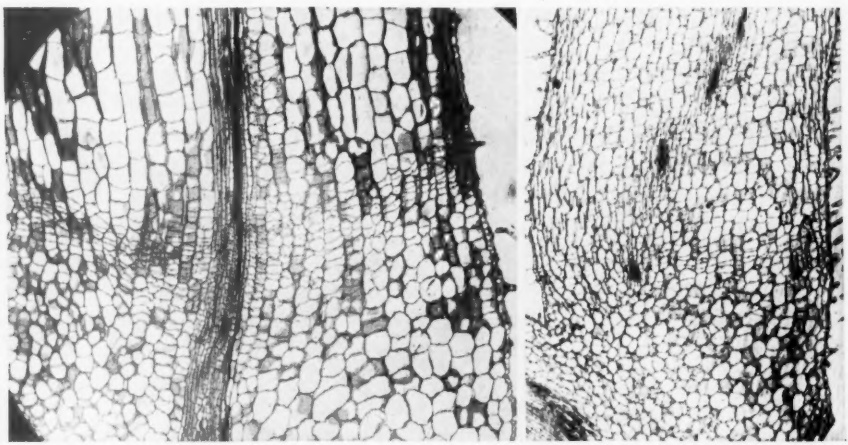
DEVELOPMENT OF ABSCISS LAYER AND ABSCISSION

Under ordinary conditions the loss of leaves of coleus probably is always the result of two processes: growth of an absciss layer, followed by dissolution of certain lamellae in the cell walls in this layer. Under certain conditions, either process may occur without the other.

The absciss layer is formed at the base of the petiole in a plane normal to the opposed leaves. It usually begins development in the third pair of leaves and matures in the sixth and seventh pairs. Its initiation is first evident by the appearance of a few transverse divided cells in the epidermis and periphery of the cortex. In a short time it appears as a ring of dividing cells around the base of the petiole. These cells continue to divide, and as more cells become involved the ring gradually widens and thickens, finally extending across all the tissues of the petiole except the xylem. Ten to fifteen layers of cells may be involved in the

outer part, but only six to eight layers in the central part of the petiole. The absciss layer, however, is about the same thickness throughout, since the cells are of different sizes.

When sections are cut at right angles to the plane of the absciss layer it has the appearance of a meristem. The cell contents stain darker than the other cells in the cortex, have more prominent nuclei, and lack large vacuoles. As it nears maturity the cell contents become granular and the cell walls less distinct. At maturity it becomes externally evident by the slight depression in the petiole (the



FIGS. 2, 3.—Fig. 2 (left), longisection through base of petiole of sixth pair of leaves below terminal bud, showing mature absciss layers. Fig. 3 (right), same of petiole of first pair of leaves below terminal bud; leaf debladed 3 months previously.

abscission zone), since the cells on either side of it continue enlarging while those in the absciss layer divide with but little enlargement.

In the younger leaves the cells in the region where the absciss layer will later form are indistinguishable from the other cortical cells of the petiole. Beginning with the third pair of leaves below the terminal bud, however, a distinct difference becomes evident. The cells of the petiole distal to the absciss layer become much larger and more elongated than those of the abscission zone and stem cortex. An absciss layer that is mature is shown in figure 2.

The final step in the separation of the leaf from the stem is the dissolution of a portion of the walls of some of the cells in the absciss layer. Shortly before abscission takes place, the petiole—and eventually the entire leaf—begins to lose its green color, and at the time of abscission it is usually pale yellow. When a break in

the lower part of the base of the petiole is evident the abscission processes are well advanced, and in a few days separation is completed.

Usually disintegration of cell walls is evident in two or three layers in the distal part of the absciss layer. The process apparently begins in the cells in the lower side of the petiole. As the middle lamellae of these cells dissolve, the longitudinal shrinkage of the cortex pulls them slightly apart and their exposed surfaces round up as a result of turgor. Once the first break is made the dissolution continues rather rapidly. Dissolution continues through the absciss layer and gradually extends through the epidermal cells and through the cortex, until all that supports the petiole is the upper part of the cortex and xylem. Finally these cortical cells separate, the xylem ruptures, and the leaf falls.

Effect of removing the blades

Investigators (**3, 4, 7, 19, 15, 18, 20, 21, 22, 23**) have shown that abscission is accelerated by high and low light intensities, different lengths of day, high and low water supply, high and freezing temperatures, low concentrations of anesthetics, toxic concentrations of acids and salts, ethylene gas, wounding of and complete removal of the blade.

To follow the internal changes that occur after deblading and to compare these changes with those occurring in an intact leaf, all the blades of one group of plants were removed and in a second group one blade of each pair of leaves was removed, the opposite leaf remaining intact. Sections cut through the absciss layers of the younger leaves usually indicated that cell division of debladed petioles had been accelerated. In the fifth and lower pairs of leaves acceleration of cell division was not so evident. In these older leaves the absciss layers were mature or almost so, and deblading did not cause additional cell division. The deblading, however, always accelerated abscission of the petioles.

There were several variations. Occasionally abscission occurred without development of the absciss layer. Abscission of debladed petioles of the third, fourth, and fifth pairs of leaves often occurred without further development of the immature absciss layers, beyond that of the opposed intact leaves. The cell walls in the debladed petioles were less distinct, the cell contents more granular and more readily stained—the usual conditions just prior to abscission. In the first petiole below the terminal bud, in which no absciss layer was visible at the time of deblading, abscission often took place without development of an absciss layer. It was frequently observed that these young petioles did not increase in size and did not abscise after deblading, or abscised only after more than 3 months, when they were in the same position as the sixth pair of leaves. In such petioles a well-defined absciss layer had developed (fig. 3), but dissolution of the cell wall lamellae was not taking place. The factors causing this long delay have not been discovered.

After debladding, the youngest petioles did not abscise as soon as the oldest petioles, and there was a gradient in time between them (fig. 5). At the time of debladding the abscise layers were in various stages of development, from none in the first two pairs of leaves to one that was mature in the sixth pair. Apparently the length of time before abscission under these conditions is related to the degree of maturity of the abscise layer or to the age of the leaf. Since debladding usually accelerates development of the abscise layer, it matures more rapidly in older leaves where it is already well advanced. DOUBT (3) found that treating coleus with ethylene gas caused the oldest leaves to abscise first.

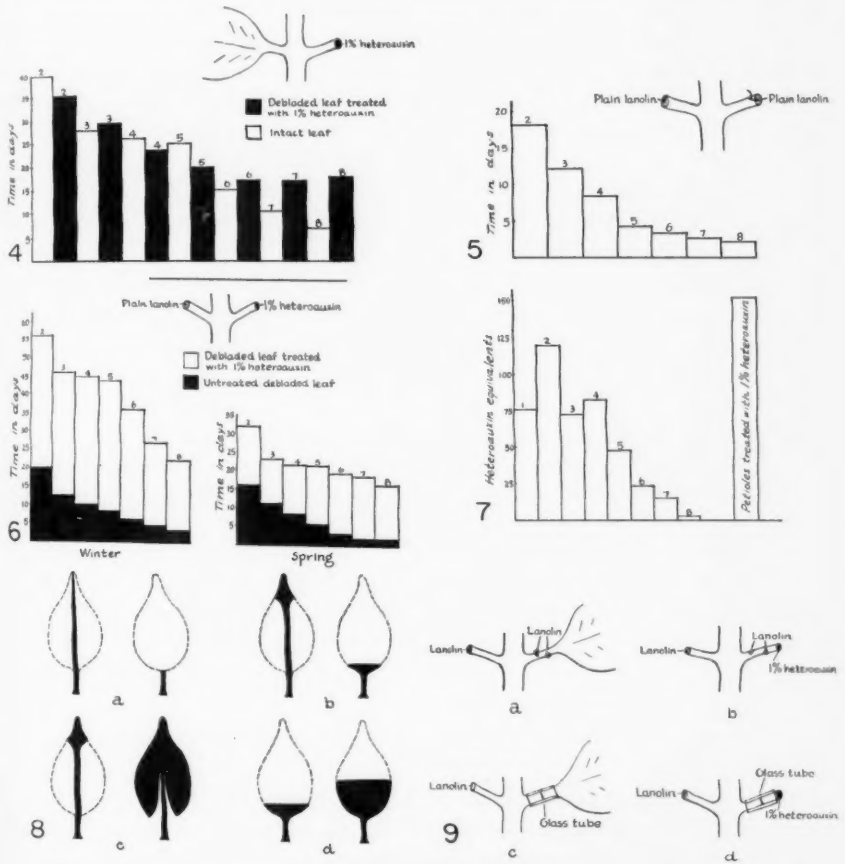
EFFECT OF GROWTH SUBSTANCES ON DEVELOPMENT OF ABSCISS LAYER AND ON ABSCISSION OF DEBLADED PETIOLES

In one group of plants a blade was removed from one of two opposite leaves at each node of the stem and a 1 per cent mixture of heteroauxin applied to the ends of the debladed petioles (fig. 4). By cutting freehand sections it was possible to compare the abscise layers and abscission phenomena in a treated debladed petiole with those in the petiole of the opposite intact leaf.

Development of the abscise layers in the treated debladed petioles was similar to that in the petioles of intact leaves. The time elapsing before abscission occurred was nearly the same (fig. 4), except in the oldest leaves which dropped 1–10 days sooner than the treated debladed petioles. As will be shown later, this fact may be correlated with the smaller amount of growth substance coming from the blades of the older leaves, indicating that heteroauxin delays the final stages of abscission.

In another experiment all the blades were removed from the plants and a 1 per cent heteroauxin mixture was applied to one petiole at each node, and the opposite petiole smeared with plain lanolin. Treatment of the debladed petioles usually delayed development of the immature abscise layers, and abscission. It retarded initiation of the abscise layer and its growth at any stage of development. The increase in the pre-abscission period depended upon the stage of development of the abscise layers and the time of year (fig. 6). The more mature the abscise layer the shorter the time before abscission. During May the abscission time was less than that for the leaves debladed in January. In the sixth, seventh, and eighth pairs of leaves the abscise layers were so well developed that there was little difference in their appearance in the treated and untreated petioles. Usually there was decided delay in the growth of the abscise layers in the young petioles, but occasionally no difference could be seen. These facts indicate that either the heteroauxin did not always delay development of the abscise layer, or the development of the abscise layer was not always accelerated by removal of the blade.

Deblading a leaf stopped elongation of the petiole. Diameter measurements were not made, but it was apparent that there was little or no increase in diameter.



FIGS. 4–9.—Fig. 4, pre-abscission period of pairs of untreated debladed petioles; numbers refer to position of leaves below terminal bud. Started March 2. Fig. 5, same of treated debladed petioles and opposed intact leaves. Started May 2. Fig. 6, same of treated debladed petioles and opposed untreated debladed leaves. Started in January and in May. Fig. 7, amount of growth substance diffusing from leaves and petioles treated with 1.0 per cent heteroauxin; tests carried out in March. Average of 10 tests. (In gammas of heteroauxin equivalents; controls with agar containing 100 gammas of heteroauxin per liter.) Fig. 8, method of treating leaves (dotted lines indicate portion removed). Fig. 9, method of treating leaves and petioles.

A treated debladed petiole, however, usually elongated as much as the petioles of the opposite intact leaf. Likewise the increase in diameter was similar. MAI (16)

had similar results when he treated debladed petioles with pollen. The amount of enlargement depended on the age of the leaf. Many of the younger petioles continued to elongate and reached or exceeded the dimensions of the petioles of the opposite intact leaves. The petioles of the first leaves below the terminal bud did not enlarge as much as the petioles of the second and third leaves. There was little elongation of the older petioles, but there was usually more than that in the opposite intact leaves.

These experiments indicate that the heteroauxin has an effect on elongation of the petioles, development of the absciss layers, and on abscission of the petioles similar in kind to that of the blade and nearly equal to it in magnitude.

In other experiments concentrations of 1, 0.5, and 0.1 per cent heteroauxin were used. The 1 and 0.5 per cent concentrations were about equally effective in delaying abscission but about 25 per cent more effective than the 0.1 per cent concentration.

Other growth substances, indolepropionic and indolebutyric acids, were applied to debladed petioles in 1 per cent concentrations. The indolebutyric acid was about as effective as heteroauxin in delaying abscission, but the indolepropionic acid was about 25 per cent less effective.

Measurements of growth substances diffusing from leaves

Measurements of growth substances diffusing from coleus leaves were made by the double decapitation *Avena* technique (**24**). The leaves were severed from the plants, the cut ends wiped with moist filter paper and attached to a sheet of agar with a drop of 0.5 per cent gelatin. At the end of 2 hours the leaves were removed, the agar allowed to stand for 40 minutes and then cut into twelve equal blocks and applied to decapitated *Avena* coleoptiles. Tests were made for the amount of phytohormones diffusing from petioles treated with 1 per cent heteroauxin on the distal ends.

The greatest amount of phytohormones diffused from the upper leaves (fig. 7). It is in these leaves that expansion occurs most rapidly. There was less diffusing from the older leaves where expansion had almost ceased. Usually there was none diffusing from the eighth pair of leaves. Avery (**2**), working with tobacco, and Goodwin (**5**) with two species of *Solidago*, had similar results. The quantity of substances diffusing from the treated petioles exceeded that coming from untreated leaves but was not much greater than that coming from the second pair of leaves during the spring.

Leaves tested during the winter and early spring gave very weak or negative tests for growth substances; during the late spring the amount increased considerably. Gustafson (**6**) had similar results with certain fruits. It is interesting to note that abscission took place more slowly during the winter when the amount of

phytohormones was low than during the late spring when it was higher, and that a 1 per cent concentration was more effective in inhibiting abscission in the summer than in the winter. Treated petioles remained on the plants much longer in the winter than in the summer, but the effectiveness of the treatment is based upon the behavior of the controls for each season.

During the winter (January to March) the second untreated debladed petioles abscised in an average of 21 days, and the treated debladed petioles abscised in an average of 57 days (fig. 6). In the spring (May to June) similar petioles abscised in 16 and 32 days, respectively (fig. 6) and in the summer (June to August) in 4 days and 30 days, respectively. The abscission of intact leaves was considerably slower during the winter than in the spring and summer. During the winter months growth of the entire plant was also much slower than during the spring and summer. It is obvious that internal conditions other than the growth substances are also primary factors in abscission.

Movement of the inhibiting effect

Experiments were designed to determine whether the inhibiting effect of the blade moved across, up, or down the stem. In one group of plants one blade of each pair of leaves was removed, the opposite leaf remaining intact, and in another group of plants both blades were removed. There was no significant difference in the time of abscission or in the development of the absciss layers in the debladed petioles of the first group and in the second group. This indicates that there is no movement of the inhibiting effect of the blade across the stem to the absciss layer of the opposite leaf, at least in significant amounts.

In another experiment all blades were removed from two groups of plants. All petioles of one group remained untreated except for a coating of plain lanolin. In another group one petiole of each pair was treated with 1 per cent heteroauxin, the opposite petiole being smeared with plain lanolin. There was little difference in the development of the absciss layers or in the time of abscission of the untreated petioles of these two groups.

One per cent heteroauxin placed on the sides of the stem of plants from which all the blades had been removed did not cause significant inhibition of the debladed petioles, either above or below the treated part. It has been shown by Snow (19) and others that under natural conditions the movement of growth substances is polar, that is, down the stem. Avery (2) showed that phytohormones moved down the leaf through the veins and midrib. Hitchcock and Zimmerman (8), however, showed that the application of certain synthetic substances in water to the soil of potted plants caused root formation on the stem, epinasty of leaves, and other effects, indicating a movement up into the stem and leaves. They were unable to secure these effects, except near the point of applica-

tion, when they used the mixture in lanolin. When cuttings were placed in a strong solution of heteroauxin (1:30,000) for 4 hours and the blades removed there was delay of a few days in the abscission of the petioles as compared with comparable cuttings in distilled water.

When approach grafts were made by splicing one of a pair of debladed petioles to an intact leaf of another plant, abscission of the grafted petioles was delayed not more than 2 days longer than the controls, although both petioles usually abscised in about the same time. This may indicate some movement of plant hormone across the graft. No grafts formed a union.

These data indicate that there is usually no significant movement of the inhibiting effect of the blade or of applied substance across or up or down the stem. When cuttings are placed in a strong heteroauxin solution there is apparently some movement up the stem and into the petioles.

Later evidence will show that the inhibiting effect of applied hormone, and presumably also of the blade, is correlated with the amount of substance translocated. Further evidence will be given indicating that under certain conditions there is no significant movement of the inhibiting effect from an intact leaf to the opposite intact leaf.

Effect of placing plants in continuous darkness

Zimmerman and Hitchcock (**25**) found that tomato plants kept in the dark for 5 days did not show geotropic effects, but curved down when heteroauxin was applied to the stem. They mentioned that if the assumption of Keeble *et al.* (**10**)—that the upward growth of plants placed in a horizontal position is due to a redistribution of substance—is correct, plants that no longer grow upward should not contain growth substances.

Avery (**2**) reported that phytohormones disappeared from tobacco plants after 5 days in the dark. Zimmerman and Hitchcock (**25**), however, reported that tobacco plants 18 inches tall still elongated after 30 days in the dark. They found it difficult to account for this continued growth if there can be "no growth without the presence of hormones." Using plants with storage organs (potatoes), they obtained geotropic effects and stem elongation after more than 60 days in the dark. Starch and sugars were detected as long as growth continued.

Rapidly growing coleus plants were placed in a dark box in June. At the end of about 10 days the leaves began to abscise, the youngest and oldest leaves abscising first. No absciss layers were found in the upper two pairs of leaves at the time of abscission. In the older leaves there was apparently some acceleration of these layers. Abscission of intact leaves was delayed by the application of heteroauxin 1–10 days longer than the opposite intact untreated leaves. There was usually more elongation of the petioles and more increase in area of the blade of the

younger intact treated leaves than in the opposite intact leaves. The untreated plants had lost most of their leaves after 21 days, but the stem and remaining petioles were still elongating and grew upward when placed in a horizontal position. Application of growth substances also caused bending of the stem. Shortly after this the plants began to rot and the experiment was discarded. Decay usually took place at the tip of the stem and in leaves that had not abscised. It affected entire leaves but ceased at a point where the absciss layer usually formed. It did not involve any stem tissue until later.

Coleus plants were placed in shaded parts of the greenhouse. One of each pair of leaves at the five lower nodes below the terminal bud was inclosed in a black sateen bag,[2] the opposite leaves being inclosed in similar white bags. The leaves in the black bags abscised 1–4 weeks before the leaves in the white bags. Other plants with the leaves similarly inclosed in white and black bags, and not in either type of bag, were placed in a dark box. The leaves of these three groups of plants abscised in about the same time. These data indicate that the blade is most effective in delaying abscission when it is exposed to light. The effect is not translocated from a leaf in the light to the opposite one inclosed in a black bag in sufficient quantities to cause a significant delay in abscission.

Effect of removing certain portions of the blade

La Rue (**12**) reported that as little as one-fourth of the blade of a coleus leaf left intact was sufficient to inhibit abscission of the petiole, but he did not indicate the part of the blade removed or the extent of the inhibition. In a series of experiments to check the relative effectiveness of different parts of the blade, various parts and amounts were removed. Plants bearing seven pairs of leaves were used in each experiment:

1. At each of the five lower nodes one leaf of each pair was debladed (fig. 8*a*). At each of the five lower nodes, therefore, there was a debladed petiole opposed by a petiole plus the midrib. A narrow ridge of the blade left attached to the midrib of the younger leaves expanded considerably, so the two upper pairs of leaves were not used. The pre-abscission period of petioles bearing the midribs was usually 1–2 days longer than the debladed petioles.

2. All the blade except a small portion of the tip was stripped from the midrib of one leaf at each of the five lower nodes. All the blade of the opposed leaf was removed except a portion at the base of the blade about equal in area to the tip of the opposite leaf (fig. 8*b*). At each node, therefore, a leaf with only the extreme tip of the blade intact opposed a leaf with a small portion of the base of the blade intact. The base of the blade delayed abscission $1\frac{1}{2}$–6 days longer than the tip

[2] A Weston light meter placed inside the bags gave no reading when held at a distance of 10 inches from a 60-watt bulb.

of the blade. The areas of the opposed leaves were equal only at the beginning of the experiment. The tips did not expand, but there was some expansion of the bases, especially of the younger leaves.

3. Incisions were made through the blade on each side of the midrib of a leaf at each of the five lower nodes. These incisions extended from the base of the blade almost to the tip, leaving an area at the end of the blade undisturbed and with the lower part of the blade detached from the midrib but connected to the tip of the blade (fig. 8*c*). The blades of the opposed leaves were removed from the midrib except for an area at the tips about equal to that of the undisturbed portion of the opposite leaf. The tip of the blade plus the rest of the blade detached from the midrib delayed abscission longer than the tip of the blade plus the midrib. The difference was less than 1 day for the older leaves to 5 days for some of the upper leaves.

4. One-half of the upper part of the blade of a leaf at each of the five lower nodes was removed. About seven-eighths of the upper part of the blade of the opposite leaves was removed, so that at these nodes a leaf with one-half the blade intact opposed a leaf with one-eighth the blade intact (fig. 8*d*). The larger portion of the blade delayed abscission longer than the smaller portions, the difference ranging from 6 to 8 days.

These data indicate that the base of the blade is more effective than the tip in delaying abscission. The midrib is not very effective. One-half the blade will delay abscission longer than one-eighth of the base of the blade.

Effect of certain treatments of the petioles

The following experiments were set up to compare the movement of the effect of the blade in delaying abscission with the movement of the effect of heteroauxin in delaying abscission:

1. Two deep incisions were made in the upper and lower sides of the petiole of a leaf at each node of the five lower nodes of coleus plants (fig. 9*a*). These incisions cut through most of the petiole's tissue and left only a narrow bridge of tissue connecting the two parts of the petioles and completely severed the semicircular vascular system. The blades were therefore attached to the petioles by a bridge of cells but without direct vascular connections. Lanolin was rubbed into the incisions and the leaves were supported in the usual position with a piece of tinfoil wrapped around the petioles. Usually the movement of water from the stem and leaves was greatly reduced by these incisions and the leaves soon wilted. The blade of the opposite leaf was removed. The debladed petioles abscised 2–10 days sooner than the leaves with the incised petioles. Some of the younger leaves par-

tially recovered and occasionally six to eight adventitious roots grew from the petiole, just distal to the upper incisions.

2. In a similar experiment all the blades were removed from a plant. Two deep incisions as described in (1) were made in a petiole at each of the five lower nodes. The distal ends of these petioles were treated with heteroauxin (fig. 9*b*); the opposite petioles left untreated. The debladed petioles abscised 3–14 days sooner than the opposed treated petioles.

3. The blades were severed from a leaf at each of the five lower nodes. These blades were then replaced on the stump of the petiole and held in place with a piece of glass tubing filled with lanolin or melted agar. The tube fitted tightly over the two portions of the petiole and held them close together (fig. 9*c*). The opposed petioles were debladed. There was no significant difference in the preabscission period of the debladed petioles and the petioles with the blade severed and replaced.

4. An experiment similar to (3) was set up with heteroauxin substituted for the blade. All the blades were removed from each of the five lower nodes. One petiole at each node was severed in the middle and replaced on the stump and held in place with a piece of glass tubing filled with lanolin or agar. The distal end of the petiole was coated with heteroauxin (fig. 9*d*). There was no significant difference in the time of abscission of the opposed petioles.

These data indicate that the movement of the effective agent of the blade in delaying abscission is similar to the movement of heteroauxin substituted for the blade, because: (*a*) the effect of the blades moves through the cells of the petiole when the vascular system is severed and the leaves wilted; (*b*) the heteroauxin delays abscission when it is applied to the end of a debladed petiole that has the vascular system severed; (*c*) the effect of the blade does not move across a cut surface in significant amounts, as a blade severed and replaced on the petioles does not delay abscission; and (*d*) the heteroauxin does not move across a cut surface in a debladed petiole that has been severed and replaced on the petiole, in sufficient quantities to delay abscission.

Summary

1. Following the reports of others that growth substances and materials containing them delay abscission of debladed petioles, a study of their effects in relation to several other factors that affect abscission was made.

2. Growth of the coleus leaf is basipetal.

3. The blade is 85–90 per cent expanded before the abscis layer is initiated in the third pair of leaves and is mature in the sixth pair of leaves of plants bearing eight pairs.

4. Abscission usually occurs in the absciss layer, but under certain treatments it may occur without development of this layer.

5. Older leaves usually abscise before younger leaves, and factors that accelerate abscission cause older leaves to abscise first.

6. Removal of the blade accelerates abscission of all petioles, except the first pair of leaves. A well-defined absciss layer develops in these leaves after several months.

7. Partially debladed petioles do not abscise as soon as petioles from which the entire blade has been removed. Actively expanding portions of the blade are more effective in checking abscission than the mature parts.

8. The basal half of the blade delays abscission 7–10 days longer than the lower one-eighth of the blade, but not so long as the intact blade.

9. A greater amount of phytohormones diffuses from petioles of the upper leaves than from the lower leaves.

10. Tests for growth substances diffusing from leaves were negative or very low during the winter.

11. The upper intact leaves and the opposite debladed petioles treated with 1 per cent heteroauxin usually abscise in about the same time. The lower intact leaves abscise 1–7 days before the treated debladed petioles, indicating that heteroauxin delays the final stages of abscission in addition to delaying development of the absciss layer.

12. Abscission is more rapid in the spring and summer than in the winter.

13. Heteroauxin applied to debladed petioles has an effect similar to the blade in delaying abscission and favoring continued development of the petioles.

14. Development of the absciss layer and abscission is accelerated in leaves of plants placed in the dark. Heteroauxin inhibits the abscission of these leaves.

15. Heteroauxin will pass through debladed petioles which have been severed except for four or five layers of outer cortical cells in sufficient quantities to delay abscission for several days.

16. The effect of the blades passes through cells of petioles severed in a similar manner and delays abscission.

17. The pre-abscission period of debladed petioles of cuttings is increased by placing them in a strong solution of heteroauxin.

18. One and 0.5 per cent concentrations of heteroauxin are about equally effective in delaying abscission but are about 25 per cent more effective than a 0.1 per cent concentration.

19. Indolepropionic and indolebutyric acids have a similar effect in delaying abscission.

20. All experiments indicate that growth substances from the blades influence the development of the abscis layer and the process of abscission, but their relation to abscission phenomena does not seem to be a simple one.

The writer wishes to thank Dr. H. C. SAMPSON for suggesting the problem, for his critical advice, and for supervising the organization of the results.

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EFFECT OF SOME ENVIRONMENTAL FACTORS ON FLORAL INITIATION IN XANTHIUM[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 517

LOUIS K. MANN

(WITH FIVE FIGURES)

Introduction

Attention has recently been called to the fact that there are effects attributable to both the dark period and the photoperiod in the initiation of flower primordia and the development of flowers resulting from photoperiodic treatment. This interpretation has been applied to the reactions of short-day plants. In the cocklebur (*Xanthium pennsylvanicum*) and the Biloxi soybean (*Soja max*), the conditions prevailing during either the dark period or the photoperiod may be a limiting factor in the induction of flowers (2). For example, *Xanthium* will flower after exposure to a 12-hour dark period following a photoperiod of high-intensity light. Similar plants will remain vegetative if the photoperiod preceding this dark period is of very low light intensity. But, regardless of the length of the photoperiod or the intensity of the light, a continuous dark period of at least $8\frac{1}{2}$–9 hours is necessary for the induction of flower primordia.

In an endeavor to provide a brief formulation of the fact that both the photoperiod and the dark period are concerned in the ultimate conditions necessary for floral initiation and development, HAMNER (2) has suggested that their relationship may be represented as follows: A, B→C, in which A represents the changes or conditions which arise owing to exposure to light; B, those owing to darkness; and C, the possible summation or resultant changes related to A and B. These same terms are used here, but it is not assumed that definite, known reactions are represented by them. The physical or chemical changes or conditions constituting A, B, and C are still to be determined. Although there is some evidence that diffusible substances are involved (3), up to now attempts to isolate flower-inducing substances have not demonstrated either their actual occurrence or their nature.

Following specific photoperiodic treatments, conditions A, B, and their possible resultant, C, may become evident through the initiation and development of flower structures. In so far as A, B, and C pertain to floral initiation and develop-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Part of the equipment was obtained through a grant to Dr. K. C. Hamner from the American Association for the Advancement of Science.

ment, the degree of initiation and development may be used as an indicator of the intensity of these conditions, although it is generally recognized that there are definite responses by other parts of the plant as well. In the following experiments conditions of light intensity and temperature were carefully controlled, and an estimate of the intensity of A and B as conditioned by these factors is assumed to be indicated by the degree of floral initiation and development.

Material and methods

Experiments 1–4 and 8 were carried out in March, April, and May, 1940, at the U.S. Horticultural Station, Beltsville, Maryland. Seeds of *Xanthium pennsylvanicum* were collected at Chicago in the autumn of 1939 and stored out-of-doors during the winter. The seeds were planted in flats, the seedlings later transplanted to $3\frac{1}{2}$-inch pots, and most experimental treatments begun when the plants—carefully selected for height and vigor—were 40 days old. They were kept on greenhouse benches receiving natural daylight, which was extended from sundown until 2:00 or 3:00 A.M. by about 100 foot-candles of Mazda filament light.

The specific treatments usually required 4–5 days for completion, after which time the plants were returned to the greenhouse for the duration of the experiment, at the end of which the stage and type of development of the terminal bud of all plants were determined by means of a low-power binocular microscope. All dissections, unless otherwise noted, were made 22 days after the conclusion of treatment. Dissections of control plants were made before and after experiments to insure that conditions of long photoperiod had been maintained; that is, that plants which received no special treatment remained strictly vegetative. The plants grew vigorously, most of them being over 3 feet high at the time the terminal bud was dissected. Because of the limited amount of soil in each pot, the plants were watered with complete nutrient solution on alternate days from the time the treatment was completed until dissection.

The character of experiments 1–4 made necessary an extensive scale for recording floral stages, the frequently used divisions into inflorescence primordia, flower primordia, etc., not being sufficiently precise to indicate the several stages recorded. The degree of initiation or development of flowers in a plant was designated by a number. The stage of floral development of strictly vegetative plants was designated as zero. In plants not strictly vegetative the number was, in general, equivalent to the transverse diameter of the terminal staminate inflorescence measured in units of 0.1 mm. For diameters below 0.7 mm. the diameter alone was not an adequate measure, and six stages—based mainly on appearance—were recognized. An inflorescence with a diameter of less than 0.7 mm. usually possesses few, if any, flower primordia, but consists for the most part of a smooth,

bulbous inflorescence axis surrounded at the base by several primordia of bracteal leaves. When an inflorescence has reached the diameter of 0.7 mm. there are usually one or more cycles of flower primordia around the basal portion of the axis. As the inflorescence continues to develop, primordia appear in acropetal succession until the axis is completely covered, a condition found in almost all inflorescences of a diameter of 1.2 mm. or larger. For any given inflorescence diameter within the range 0.7–1.2 mm., the average relative portion of the axis covered with flower primordia was determined. If in any plant with an inflorescence diameter within this range the relative portion of the enlarged axis tip covered with primordia was greatly different from the average for that diameter, the measured diameter was modified slightly. For inflorescences with a diameter greater than 1.2 mm. the diameter was used directly. Since the environment during any experiment affects initiation and rate of floral development, the stage is of comparative value and its use is justifiable only within a single experiment, or in experiments conducted under identical conditions.

For any specific treatment within an experiment, a unit which usually consisted of ten or more plants was used. When all plants of a unit were flowering (no vegetative plants present), the average of their floral stages presents a fair estimate of the resultant effects of the stimulus. If, however, a unit was given a photoperiodic treatment of just sufficient strength to bring about the first recognizable stage of flowering, the variability in the response of these plants is such that approximately half of them would show various stages of flowering while the other half would remain vegetative. Among the vegetative plants, some would require only slight additional stimulus to bring about initiation of flowers, while others would require a much greater stimulus. Since these vegetative plants are all given the value zero as their stage of flowering, no negative values being used, the numerical average of the plants in the unit is much too high. As another example, suppose that following a given photoperiodic treatment one plant flowers out of a group of ten, the remainder being vegetative; the average stage of flowering of this group would be positive and equal to one-tenth the flower stage of the single flowering plant. Rather than make an arbitrary correction for these vegetative plants in the following graphs, a list of all units containing two or more vegetative plants is given in each case.

The temperature of the greenhouse was not allowed to go below 70° F., and only on a few days in May did it rise above 85° F. All experimental rooms except the darkroom were refrigeration rooms, where light intensity, temperature, and humidity were controlled. The darkroom was not refrigerated but for most experiments was maintained at 23° C. or slightly above. Each refrigeration room contained either a 60 or a 75 ampere arc light burning Everready Sunshine car-

bons. This was supplemented by Mazda filament light of between 100 and 200 foot-candles' intensity. Light intensity was controlled by shading the arc lights with thin cloth curtains and by changing the position of the benches on which the plants were placed. Temperatures were kept within 1° C. of that specified. Relative humidity was kept within 80–100 per cent.

In several of the experiments determinations of the effect of temperature, light intensity, and other factors during a single photoperiod were made. In such cases some method must be used to remove, or at least to reduce, the effect of the light which the plants previously received in the greenhouse. It has been shown (2) for *Xanthium* that exposure to repeated short cycles, each consisting of 2–3 hours of darkness followed by 2–3 minutes of medium light intensity, has this effect. Several hours of this treatment following a long bright photoperiod lowers the effectiveness of the photoperiod to the extent that the plants may be exposed to a long dark period and no initiation of flowers will result. In the experiments presented here, except as otherwise specified, a single cycle of this treatment consisted of a 1-hour dark period followed by a 3 ± 1-minute photoperiod. The light intensity during the photoperiod was 40 foot-candles; the temperature during the entire cycle was 23° C. Exposure to several such consecutive cycles is here designated as short-cycle treatment. The cycles were usually repeated consecutively for a period of 40 hours. In some cases the control units used to test the effectiveness of the short-cycle treatments did not consist entirely of vegetative plants. However, the residual effects from previous photoperiods are lowered sufficiently so that they are probably of little significance.

As has previously been demonstrated, and as is shown in experiment 6, the duration and intensity of the light of the photoperiod immediately following a long dark period have pronounced effect on subsequent flower initiation and development. This factor was therefore controlled as much as possible. Immediately following all long dark periods the plants were placed under uniform light and temperature. Here they were exposed to a photoperiod of at least 8 hours. Following this, the plants were taken out during a period of daylight and placed on the long-day bench of the greenhouse. If, as frequently was the case, the 8-hour photoperiod did not end during daylight, it was extended until 9:00 A.M. or later of the following day, at which time the plants were removed to the greenhouse.

Experiments 5–7 were carried out at Chicago during the summer of 1939. Here the procedure was variable and will be given under each experiment. The recorded stages of floral development do not correspond with that used in experiments 1–4 and 8, but follow that previously used by HAMNER (2).

Procedure and results

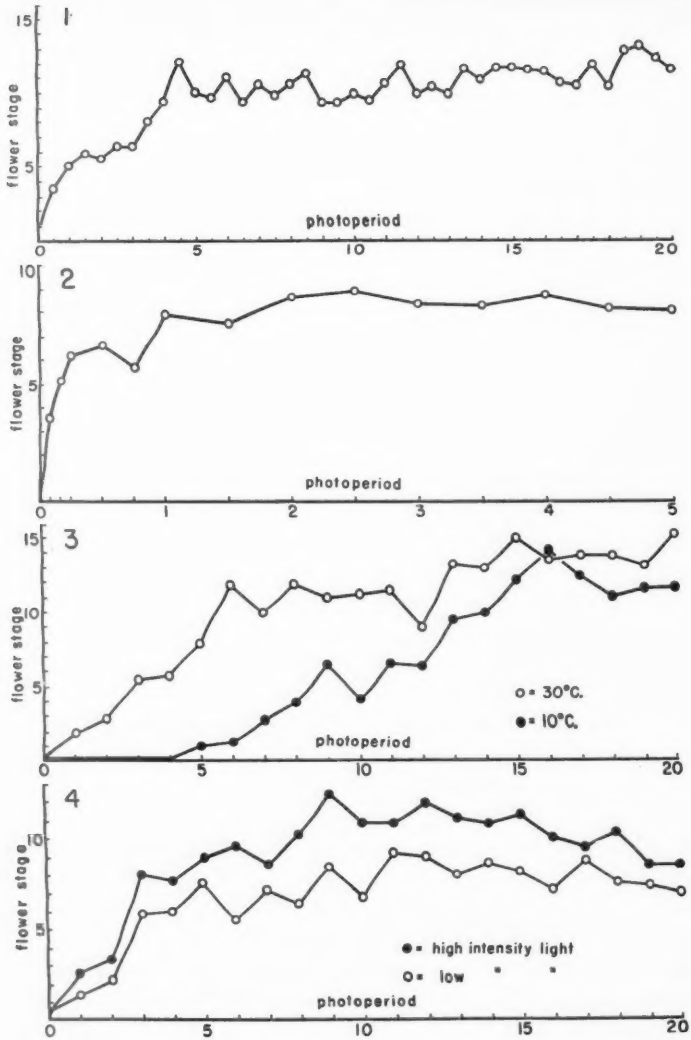
Experiment 1: Initiation and development of flowers affected by length of single photoperiod preceding long dark period

Plants of *Xanthium* were given an experimental treatment consisting of a single controlled photoinductive cycle, during which induction of flowers might take place. Previous to this controlled cycle, the plants were given a short-cycle treatment. During the single photoinductive cycle all factors remained constant except the length of the photoperiod, which was varied from ½ to 20 hours. This photoperiod, regardless of length, was followed by a 12-hour dark period.

In accordance with the general outline for experiments given before, 410 plants of a seeding of February 12 were selected from the long-day bench and placed in a control room at noon, March 12. In order to reduce the effect resulting from exposure to light in the greenhouse, the entire lot received a short-cycle treatment lasting 40 hours. Immediately following this, ten of the plants (controls) were placed in the darkroom for 12 hours. The remaining 400 plants were exposed to light of 2000–3000 foot-candles at the leaf surface, with a uniform temperature of 23° C. One-half hour later, and at each ½-hour interval thereafter, a unit of ten plants was transferred to the darkroom, 20 hours being required to remove all 400 plants. Thus the units received photoperiods ranging from ½ to 20 hours. Any given unit remained in the darkroom for 12 hours, after which time it was returned to the arc room for 8 hours or more so that it could be replaced on the long-day bench during daylight. Twenty-two days after all plants had been returned to the bench, they were harvested, the terminal buds examined, and the stage of flowering recorded.

The results of this experiment are shown in figure 1. The length of the variable photoperiod following the short-cycle treatment and preceding the 12-hour dark period is indicated by the abscissa. The average of the stage of flowering of each unit is indicated by the ordinate, which may also be interpreted as indicating the intensity of condition A as affected by the length of the single photoperiod shown on the abscissa. In the lower lefthand corner is indicated the average stage of flowering of the control unit, which received no photoperiod following the short-cycle treatment or preceding the 12-hour dark period. In this unit one plant was flowering and nine were strictly vegetative. Of the other units of the experiment, none contained more than one vegetative plant.

The average of the stage of flowering increases rapidly with the increasing length of the photoperiod up to 4–5 hours. Beyond this, increasing the length of the photoperiod has much less effect on the initiation and development of flower primordia.



FIGS. 1–4.—Effect of varied lengths of photoperiod (single photoinductive cycle) on initiation and development of flower primordia in *Xanthium:* Fig. 1, of medium light intensity; fig. 2, of high light intensity; fig. 3, at two temperatures; fig. 4, at two light intensities.

Experiment 2: Repetition of experiment 1, using photoperiod of high light intensity

This experiment was designed to determine the effect of the length of a single photoperiod preceding a long dark period on the initiation and development of flowers. The procedure differed in several respects from that of experiment 1.

Experiment 1 showed that a single $\frac{1}{2}$-hour photoperiod of moderate light intensity preceding a long dark period is sufficient to bring about the initiation of flower primordia. In experiment 2, in which a very high light intensity photoperiod was used, several photoperiods shorter than $\frac{1}{2}$ hour were given. Another precaution was also taken. The ten control plants in experiment 1 contained one which developed primordia, indicating that there still remained a measurable effect owing to the photoperiod to which the plants had been exposed while on the long-day bench. To minimize this effect still further, the type of short-cycle treatment usually given was considerably modified.

On May 1, 267 uniform plants were selected from a seeding of March 26, which had been growing on the long-day bench, and were placed in a room where they received Mazda filament light of about 5 foot-candles for 18 hours. This treatment was followed by cycles consisting of a 2-hour dark period followed by a photoperiod of 3 ± 1 minutes of 5 foot-candles of Mazda filament light. This type of treatment was continued for 73 hours at a temperature of 19°–20° C. Immediately following this treatment, one unit of eighteen plants (controls) was taken to a darkroom, avoiding exposure of these plants to light. This unit remained in the darkroom for 12 hours, when it was taken directly to the long-day bench. The remaining plants were divided into units of eighteen plants each. These were given varying lengths of photoperiods by exposure to direct sunlight outside the greenhouse. The light intensity remained above 10,000 foot-candles throughout the exposures; and since these were given during the early part of the day, the temperature rose from 15° C. at the beginning to 22.5° C. at the conclusion of the longest exposure. The photoperiods varied in length from 5 minutes to 5 hours (fig. 2). At the conclusion of any photoperiod, the unit was placed in a darkroom where it remained for 12 hours. After receiving the dark period, the unit was returned to the long-day bench, where all plants remained until dissected.

The data are given in figure 2. The abscissa and ordinate are similar to those in figure 1, except that the length of some of the photoperiods is different. The stage of flowering of the control unit, which received no photoperiod preceding a 12-hour dark period, is shown in the lower lefthand corner of the graph. All plants of this unit were strictly vegetative. Among the other units represented on the graph, that exposed to a 5-minute photoperiod contained five vegetative plants

out of eighteen, and the unit exposed to a 10-minute photoperiod contained three out of eighteen. No other lots contained more than one.

For a single photoperiod of high light intensity under the conditions of this experiment, the stage of flowering as here measured increased rapidly with increase in the length of the photoperiod up to about 2 hours. Increasing the length beyond 2 hours only slightly affected the stage of flowering of the plants.

EXPERIMENT 3: INITIATION AND DEVELOPMENT OF FLOWER PRIMORDIA AFFECTED BY TEMPERATURE DURING SINGLE PHOTOPERIOD PRECEDING LONG DARK PERIOD

Xanthium which had been grown under long-day conditions was submitted to only one photoinductive cycle, during which induction of the flowering condition might take place. As in experiment 1, preceding and following this treatment the plants were grown on the long-day bench. To reduce the effect of the photoperiods previous to the controlled photoinductive cycle, short cycles were employed. To determine the effect of temperature during the photoperiod, plants were placed in control rooms at two different temperatures, where they received photoperiods varying from 1 to 20 hours.

From plants seeded February 20 and grown under conditions of long day, 410 plants were selected. At noon, March 29, these were placed in a control room and exposed to short-cycle treatment for 40 hours. Immediately following this treatment a unit of ten plants (controls) was placed in the darkroom. The remaining 400 plants were divided into two groups of 200 each, and each group was placed in one of two control rooms, one maintained at 30° C. and one at 10° C. In both rooms the light intensity was 2000–3000 foot-candles at the leaf surface. Beginning the first hour after the plants were placed in the light rooms, units of ten plants were removed hourly from both rooms to the darkroom. Any given unit remained in the darkroom for 12 hours, after which it was returned to a control room maintained at 23° C. and having 2000–3000 foot-candles of light. After 8 hours or more in this control room the units were removed during the day to the long-day bench, where they remained until dissection.

Figure 3 gives the results of this experiment. The stage of flowering of the control unit of nine plants (one died) is shown in the lower lefthand corner of the graph. Two of these nine plants showed the first stages of flowering; the remaining seven were strictly vegetative. Of the other units in which there was more than one vegetative plant, the following list gives the lengths of the photoperiods to which they were exposed and the number of vegetative plants in each case. Among the units at 30° C., the following showed more than one vegetative plant: 1 hour, six vegetative; 2 hours, four vegetative; 3 hours, three vegetative; 4 hours, three vegetative; 5 hours, three vegetative. Among the units at 10° C., the following

contained more than one vegetative plant: 1 hour, nine vegetative; 2 hours, nine vegetative; 3 hours, all vegetative; 4 hours, all vegetative; 5 hours, three vegetative; 6 hours, three vegetative; 7 hours, three vegetative; 10 hours, five vegetative; 12 hours, two vegetative.

At a temperature of 30° C. the response of the stage of flowering to the length of the photoperiod is much like that found in experiment 1; there is a marked rate of increase in the stage of flowering with increase in the length of photoperiod up to about 6 hours. Lengthening photoperiods beyond this increases their effectiveness only slightly. At a temperature of 10° C. a photoperiod shorter than 4–5 hours had little effect so far as initiation and development of flowers were concerned.

The ordinate in figure 3 may also be interpreted as an indicator of the intensity of condition A developed by photoperiods of different lengths and at two different temperatures. In this case the level of condition A rose much more slowly in plants exposed to a photoperiod at 10° C. than in plants exposed to a photoperiod at 30° C. At 10° C., with the light intensity and quality used in this experiment, exposures to light must be 13 hours or more to bring A to the same level reached during a photoperiod of about 6 hours at 30° C. It is evident that, at least for *Xanthium*, the temperature during the photoperiod of a photoinductive cycle has a pronounced effect on subsequent initiation and development of flowers.

Experiment 4: Initiation and development of flowers affected by intensity of light during single photoperiod preceding long dark period

Following the procedure of experiment 3, the selected plants were exposed to a single photoinductive cycle during which induction of flowers might take place. Preceding and following this cycle the plants were grown under conditions of long photoperiod. The plants were divided into three lots, which were placed in separate rooms, each being exposed to a different light intensity during the single controlled photoinductive cycle. In each room the respective lots were exposed to photoperiods ranging from 1 to 20 hours. The final stage of flowering of any given unit is thus a function of the length of the photoperiod and of its light intensity.

On April 22, 610 plants of a seeding of March 13 were selected from the long-day bench of the greenhouse. To reduce the effect of the previous photoperiods, the plants were placed in a control room where they received 40 hours of short cycles. Following this treatment one unit of ten plants (control) was taken immediately to the darkroom, where it remained for 12 hours. The remaining 600 plants were divided into three lots of 200 each, which were distributed to the three arc-light rooms, all of which were kept uniformly at 23° C. In each room the plants were exposed to a different intensity of light at the leaf surface: 115–170 foot-candles in one room, 500–680 in another, and 800–1300 in the third. A unit of

ten plants was removed from each room 1 hour after the plants had been placed therein, and each hour thereafter, 20 hours being required to remove all the plants. Each unit removed was placed in the darkroom and left there for 12 hours, after which it was returned to a control room maintained at 23° C., where it received 2000–3000 foot-candles at the leaf surface. After remaining in this control room for 8 hours or more, the plants were returned to the long-day bench during daylight, where they remained until dissected.

The results of a part of this experiment are given in figure 4. The abscissa indicates the length of the variable photoperiod in hours, and the ordinate, the average of the stage of flowering for the various units of ten plants. Except for a more rapid increase during the first 2 hours of exposure, the stage of floral development of the plants exposed to the photoperiod of highest light intensity was not greatly different from that of those exposed to the medium intensity. For this reason the data for the high intensity are not shown in the graph.

The control unit, the stage of flowering of which is shown at the extreme lower lefthand corner of the graph, contained one flowering and nine vegetative plants. Of the other units containing more than one vegetative plant, the following list gives the length of photoperiod and the number of vegetative plants in each case. At the low intensity there were: 1 hour, eight vegetative; 2 hours, four vegetative; and 6 hours, two vegetative. At the medium intensity there were: 1 hour, three vegetative (nine plants in all); and 2 hours, four vegetative.

The stages of floral development resulting from exposure to these two light intensities are well separated for all photoperiods of the same length; that is, the level of condition A resulting from exposure to the lower intensity shows no tendency to approach the level at the higher intensity, even after prolonged exposure to light.

Figure 4 indicates, as do figures 1 and 3, that the stage of flowering, or the intensity of condition A, increases rapidly with increase in the length of the photoperiod for the first few hours of exposure to light, after which further increase in the photoperiod increases the stage of flowering only slightly. Further, although different light intensities may produce marked differences in the level of A, within wide ranges of light intensity this difference is relatively small.

EXPERIMENT 5: INITIATION AND DEVELOPMENT OF FLOWERS AFFECTED BY LENGTH OF SINGLE DARK PERIOD FOLLOWING LONG PHOTOPERIOD OF HIGH LIGHT INTENSITY

Xanthium may be kept indefinitely vegetative if exposed to photoperiods of 18–20 hours with intervening dark periods of 6–4 hours. If one of these cycles is modified by increasing the length of the dark period beyond $8\frac{1}{2}$–9 hours, the plants are induced to flower even though subsequently they may be returned to conditions of long photoperiod or continuous light.

For dark periods around 23° C. the minimum length of the dark period necessary to induce flowering is $8\frac{1}{2}$–9 hours. It has been shown (experiment 8) that several photoinductive cycles with long dark periods are more effective in the induction of flower primordia—and especially in the development of flowers—than a single cycle. That the effectiveness of a single long dark period might be augmented by increasing it beyond the $8\frac{1}{2}$–9 hours' minimum length necessary to induce flowering is a possibility.

This experiment was carried out at Chicago during the summer of 1939. The plants were grown on the long-day bench from June 2 until June 30. Following a bright day, units of ten plants each were taken from the long-day bench and given dark periods of various lengths, as indicated in figure 5. Following this dark period the units were exposed to either of two conditions: part of them were returned to

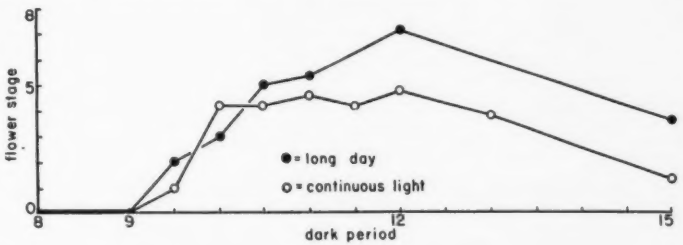


FIG. 5.—Effect of length of single dark period and subsequent photoperiodic treatment on initiation and development of flower primordia in *Xanthium*. Plants received single photoinductive cycle.

the long-day bench and part were placed under continuous light (daylight extended by Mazda filament light of about 100 foot-candles from sundown to sunrise). As indicated by unpublished experiments, and as found by HAMNER (3), long photoperiods and short dark periods following flower induction are more favorable to flower development than is continuous light.

The results of this treatment are shown in figure 5. The abscissa indicates the length of the single long dark period to which the plants were exposed; the ordinate indicates the relative amount of initiation and floral development. One line in the graph represents those plants returned to long-day conditions to develop; the other line, those which remained on continuous light. As noted under the section on methods, the scale for stage of flowering used here differs from that in experiments 1–4 and 8.

Each experimental unit consisted of ten plants. Under the conditions of this experiment all plants given dark periods of $8\frac{1}{2}$–9 hours remained strictly vegetative. Under usual experimental conditions, at least some plants receiving a dark period of this length would flower. This and the relatively large numbers of vege-

tative plants listed below probably resulted from the removal of the plants from the dark period into early morning light of rather low intensity (see experiment 6). Among those units returned to long-day conditions after treatment, the following contained more than one vegetative plant: 9.5 hours, six vegetative; 10 hours, four vegetative; 15 hours, four vegetative. Among those lots returned to continuous light after treatment, the following contained more than one vegetative plant: 9.5 hours, seven vegetative; 10 hours, two vegetative; 11 hours, three vegetative; 11.5 hours, three vegetative; 13 hours, two vegetative; 15 hours, three vegetative.

This experiment has been repeated twice, employing dark periods up to 50 hours and allowing the plants to develop on long photoperiods. Both repetitions indicate a maximum effective length of 12–15 hours for the dark period.

Experiment 6: Initiation and development of flowers affected by photoperiods of low light intensity immediately preceding and following long dark period

For *Xanthium* plants kept continuously vegetative by growing under conditions of long day, the length of the dark period is the main factor limiting the induction

TABLE 1

Effect of low light intensity on initiation and development of flowers

Treatment		Average stage of flowering	No. of plants	
Photoperiod preceding 12-hour dark period	Photoperiod following 12-hour dark period		Veg.	Fl.
12 hours of 200 f.c. Mazda filament light	12 hours of 200 f.c. Mazda filament light	0.0	20	0
	12 hours of 1200 f.c. arc light	0.3	18	2
12 hours of 1200 f.c. arc light	12 hours of 200 f.c. Mazda filament light	0.15	16	4
	12 hours of 1200 f.c. arc light	3.5	0	20

of the flowering condition. Under usual growing conditions the lengthening of a single dark period beyond $8\frac{1}{2}$–9 hours will bring about initiation. With more careful consideration of the rate of development of primordia, other factors affecting the relative strength of a photoinductive cycle are found in addition to the length of the photoperiod or dark period. A number of these have been indicated in the previous experiments. The intensity of the light of the photoperiod immediately preceding and/or following a long dark period is of considerable importance.

Selected plants were given a single dark period of 12 hours preceded and followed by 12-hour photoperiods of controlled light intensity. On October 7 eighty plants from the long-day bench were divided into two lots of forty each, one lot receiving Mazda filament light at an intensity of 200 foot-candles and the other re-

ceiving arc light of about 1200 foot-candles, for 12 hours; then each lot received a 12-hour dark period. At the end of this dark period, half the plants in each of the two treatments were returned to the light of 200 foot-candles for 12 hours, and the remaining half were placed under the light of 1200 foot-candles. At the end of this treatment the four groups were returned to the long-day bench, where they remained until harvested for dissection, 27 days after the start of the experiment. The method of measuring the flower stage in the following data is different from that used in experiments 1–4 and 8. The nature of the treatments and the data are summarized in table 1.

Experiment 7: Initiation and development of flowers by photoinductive cycles with low light intensity photoperiod

Experiment 6 indicated that decreasing the intensity of light preceding and following a long dark period markedly decreased the effectiveness of that dark period in inducing flower primordia. However, a photoperiod of very low intensity may be effective in bringing about induction of flowers if the photoinductive cycles are repeated several times.

TABLE 2

Effect of photoperiods of low light intensity on initiation and development of flowers

No. of photoperiods, each separated by 12-hour dark period	Light intensity in f.c. during photoperiod	Average stage of flowering	No. of plants	
			Veg.	Fl.
2	18	0.0	9	0
	50	0.0	10	0
	100	0.0	10	0
11	18	1.4	3	4
	50	1.6	2	8
	100	2.3	1	6

At 8:00 A.M., October 6, sixty plants were removed from the long-day bench, and two units of ten plants each were placed under each of three intensities of Mazda filament light—18, 50, and 100 foot-candles. After a 12-hour exposure, all plants received a 12-hour dark period. This dark period was followed by an exposure to 12 hours of light of the same intensity to which the unit was first exposed. Half the lots were then returned to the long-day bench, where they remained until dissected, while the remaining half received nine more of these cycles before being returned to the bench. All plants were dissected November 4. The experimental procedure and results are presented in table 2.

EXPERIMENT 8: EFFECT OF NUMBER OF PHOTOINDUCTIVE CYCLES ON RATE OF DEVELOPMENT OF FLOWERS AND FRUITS GROWN UNDER CONDITIONS OF LONG DAY

Table 3 shows in a general way the effect which the number of induction periods has on the rate of development of flower structures and fruits. The first visible evidence of inflorescence initiation appears 2–3 days following a single induction period of the type given here. For this reason the effect of the number of inductive

TABLE 3

NUMBER OF CYCLES EACH CONSISTING OF A 12-HOUR PHOTOPERIOD FOLLOWED BY A 12-HOUR DARK PERIOD. EXPERIMENT STARTED THE MORNING OF APRIL 2. ALL FIGURES WITHOUT QUALIFYING TERMS REFER TO STAGE OF FLOWERING

DATE EXAMINED	CYCLES				CONTINUOUSLY REPEATED
	1	2	4	8	
April 5	1.0	1.0	1.0	.....	.....
April 8	2.6	3.6	4.6	4.8	.....
April 11	3.3	9.1	12.3	11.6	.....
April 14	4.4	12.3	17.4	18.7	.....
April 17	7.4	13.4	23.3	33.3	.....
April 22	9.4	13.8	24.0	43.8	.....
April 24	.....	18.0	30.5	Pollen first shed	Pollen being shed
April 29	11.4	18.8	44.8	Bur length 14.2 mm.	Bur length 20.5 mm.
May 6	.....	.....	Pollen first shed	.....	.....
May 8	17.8	28.3	.....	.....	.....
June 4	Pollen being shed	Bur length 12.2 mm.	Bur length 33.3 mm.	Burs nearly mature, becoming brown	Burs completely mature, length 22.4 mm.; plants dead

cycles is much less evident in earlier stages of development of inflorescence and flower primordia than in the later stages. The later development proceeds much more rapidly in plants which received several short-day treatments than in plants which received only one or two such treatments.

Plants were selected April 2 from a group seeded on the long-day bench February 20. The cycles used for flower induction consisted of a 12-hour photoperiod of natural daylight in the greenhouse (6:00 A.M. to 6:00 P.M.) followed by a 12-hour dark period. The various lots received 1, 2, 4, 8, and continuously repeated cycles. All lots except the last were returned to the long-day bench for development after treatment. The stage of flowering is measured on the same scale as that used for experiments 1–4.

By June 4, 64 days after induction began, the plants on continuous short day were completely mature and had died. At the other extreme were those plants which had received only one long night and were just beginning to shed pollen. Plants developing on continuous short days bore fruit which differed markedly from that of plants induced with a few short days and then allowed to develop on long days. The burs of the former are much smaller, bear only a few spines (in comparison with those on long day), and frequently the thin involucre is broken, allowing the ovary to protrude for half its length.

Discussion

The effect of temperature during the dark period may be compared with that during the photoperiod. LONG (4) has shown that lowering the temperature increases the critical length of the dark period. The temperature during the dark period is also of importance in flower induction in Biloxi soybeans (5). It has been previously found with *Xanthium* that temperature had little effect on the photoperiodic reaction (4). Experiment 3, however, shows definite temperature effect. For short photoperiods this effect was pronounced, but it decreased as the photoperiods increased in length. Previous workers, when considering the relative effects of temperature on the dark period and the photoperiod, have used dark periods near the critical length but photoperiods far in excess of the minimum length. This probably explains the resultant relatively low coefficient of temperature for the reactions of the photoperiod. In Biloxi soybean the effect of temperature was found to be slight but significant (5).

In comparing the results of these experiments with other data, especially from other species, it must be kept in mind that the flowering responses in experiments 1–6 depended upon variable factors introduced into a single photoinductive cycle. The plants were exposed to only one cycle during which flower induction could have taken place. Many short-day plants, such as Biloxi soybean, require several cycles of treatment for such induction. LONG (4) has shown that when several cycles are given, the time elapsing between consecutive cycles is of great importance. This spacing may determine in part the most effective length of photoperiod or dark period for induction of flowers, as has been suggested by HAMNER (2). Thus in experiment 5 the optimum length for the dark period was found to be 12–15 hours, although this would probably be decreased if several cycles were given instead of one, since long dark periods space the photoinductive cycles farther apart. When several photoinductive cycles are given, this effect must be considered.

The importance of the light period immediately following a dark period has been neglected in most work with *Xanthium*. Experiment 6 indicates that the photoperiod following a long dark period—as well as that preceding it—may be impor-

tant. When plants are exposed to several consecutive photoinductive cycles, a photoperiod both follows and precedes a dark period. The role of the photoperiod under these conditions may be somewhat different from a single-cycle experiment, where the test photoperiod only precedes a dark period. Experimental work on the effect of light immediately following a dark period is needed.

In the preceding experiments reference has been made to condition A, which has been defined as "the changes or conditions which arise owing to exposure to light" (2). Such changes or conditions are those which develop on exposure to high light intensity at moderate temperatures. Within certain limits the intensity of A increases with increasing exposure to light. In addition to its function of establishing condition A, but probably distinct from it, light interferes with the reactions which occur during the dark period following a photoperiod. Here continuous light of very low intensity or periods of moderate intensity and of very short duration are effective. In *Xanthium*, 1 minute of light in the middle of a 9-hour dark period will nullify the effect of the dark period so far as the initiation of flowers is concerned. In Biloxi soybean, BORTHWICK and PARKER (1) have demonstrated that Mazda light of 0.6 foot-candles, given for the first 8 hours of a 16-hour dark period, will prevent flower induction.

In *Xanthium* and Biloxi soybean condition A is developed most rapidly under light of high intensity. On the other hand, development of condition B depends on the exclusion of light, 0.6 foot-candles being sufficient to inhibit this condition in the soybean. There is nothing to indicate that the reactions brought about by light of high intensity (the establishment of condition A) are the same as those which occur when light interferes with the reactions taking place in the dark.

If two distinct reactions or groups of reactions are present, the recognition of this fact is of some importance. WITHROW and BIEBEL (6), using salvia, a short-day plant, found that flowering plants would return to the vegetative state if placed on long photoperiods. Giving these plants a short photoperiod of natural daylight and extending this with additional low light intensity also caused the plants to revert to the vegetative condition; that is, the short photoperiod supplemented by low light intensity brought about a response characteristic of long photoperiods. At low intensities red radiation was found to be more effective as supplementary light than other portions of the spectrum used. They conclude that, "under the conditions of the experiment, red radiation is most effective in producing the photoperiodic response both in long and short day plants." It would seem that, with respect to short-day plants, their experiments dealt with the second mentioned response to light rather than with the establishment of condition A, and indicate that red radiation is most effective in interrupting the dark period—in interfering with condition B.

Summary

1. Induction of flowers may take place in vegetative plants of *Xanthium pennsylvanicum* when subjected to a single controlled photoinductive cycle. This flowering response is recorded on a more extensive scale than has previously been used. The measurement is based on the size of the apical inflorescence.

2. The effect of the length of the photoperiod given during this single cycle is related both to its temperature and to its light intensity. In general, the initiation of flower primordia and their rate of development increase with the increasing length of the photoperiod up to a rather definite point, beyond which further lengthening of the photoperiod has little effect.

3. By increasing the light intensity, the effectiveness of the photoperiod of the single photoinductive cycle is increased, at least for large differences of light intensity. This response seems independent of the length of the photoperiod, being evident even after 20 hours. Photoperiods of as low as 18 foot-candles may bring about induction if repeated consecutively several times.

4. The temperature during the photoperiod has a pronounced effect on the resultant stage of flowering. At 10° C. a single photoperiod of 2000–3000 foot-candles must be in excess of 4–5 hours to cause flowering. At 30° C. a photoperiod of similar intensity but only ½ hour in length may cause initiation. As the photoperiod increases in length, the effect of the temperature diminishes.

5. In plants induced to flower by increasing the length of a single dark period beyond 8½–9 hours, the maximum stage of flowering is brought about by dark periods of 12–15 hours.

6. The photoperiod following a long dark period—as well as that preceding it—has a pronounced effect on the initiation and development of flowers.

7. Data are given showing the influence of the number of photoinductive cycles to which plants are exposed on the rate of development of flower structures.

8. Some of the data of these experiments are interpreted on the basis of HAMNER's suggestion that the induction of certain short-day plants may be represented by A, B→C.

The writer appreciates the aid and encouragement of Dr. K. C. HAMNER during the progress of this work.

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EFFECT OF BILE SALTS AND OLEATES ON THE STRUCTURAL VISCOSITY OF PROTOPLASM[1]

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(WITH THREE FIGURES)

Introduction

As a result of facts derived from many investigations upon the nature of protoplasm, BAITSELL (2), FREY-WYSSLING (4), NEEDHAM (5), PETERS (6), NORTHEN (7), and others have concluded that protoplasm has a definite structure. According to SEIFRIZ (10), this continuity of structure may be regarded as the determinant of the living as opposed to the nonliving. With further knowledge concerning naturally occurring substances, the proteins in particular, the nature of this structure becomes more and more clarified.

The basic units of protoplasmic structure are conceived as being the fibrous or threadlike molecules of proteins, which through the linkages of their side chains form a network structure to which NEEDHAM (5) applied the term "cytoskeleton." PETERS (6) has elaborated this concept by distinguishing three parts: (*a*) the surface proteins; (*b*) the cytoplasmic proteins (with only slight chemical differences from the surface proteins but more important differences of orientation); and (*c*) the nuclear proteins.

Fats and fatlike substances are regarded as indispensable constituents of living tissue, with functions based upon their solubility relationships and physical as well as chemical properties. OSBORNE (9) has concluded that a chemical union, unstable toward alcohol, exists between the protein and the phosphatide of lecithovitellin of egg yolk. Experiments by MÂCHEBOEUF (9) and by SØRENSON (9) indicate that the linkage between the lipid and the protein is probably a residual or nonpolar valency linkage. It is possible that the lipids, in part, function as a cement to connect the protein molecules into a network or cytoskeleton, and have a fundamental role in dissociation reactions (12).

FREY-WYSSLING (4) has suggested the incorporation of phosphatides into the protein reticulum through heteropolar cohesion bonds, or secondary valency bonds, between hydrophilic side chains (—OH. . .HO—), and through homopolar cohesion bonds between lipophilic side chains (—CH_3 H_3C—). Likewise the fats would be associated with the protein chains directly or through phosphatide molecules by means of the homopolar cohesion bond.

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 182.

In view of such a protoplasmic structure, based upon a protein network in which fats and lipoids are incorporated, experiments were performed with substances such as sodium glycocholate, sodium taurocholate, sodium oleate, and magnesium oleate (which are known to affect fats and lipoids) to determine their effects upon the structural viscosity of protoplasm.

Method

The method of measurement of changes in structural viscosity is based upon the equation: $V = k(c - c_0)$, derived by NORTHEN (7), which approximately governs the rate with which the chloroplasts in cells of *Spirogyra* move in response to different centrifugal accelerations. In the equation, V is the velocity of chloroplastic movement, k a constant, c the centrifugal acceleration, and c_0 is the initial centrifugal acceleration at which—or below which—the chloroplasts will not move regardless of the length of time the acceleration is allowed to act.

In general, the acceleration that would displace the chloroplasts in only 5–20 per cent of the filaments was used. The different accelerations usually employed in determining this particular one were 1062×gravity, 680×gravity, 382×gravity, 245×gravity, and 170×gravity. If the acceleration were increased to the next higher value, 50 per cent or more of the filaments would have the chloroplasts displaced.

The c_0 value is not uniform for all *Spirogyra* filaments in a sample group; therefore, when a group of filaments is centrifuged with an acceleration of, for example, 680×gravity, the chloroplasts will be displaced in the cells of those filaments whose c_0 is less than 680×gravity and will not be displaced in cells with a c_0 value equal to or greater than 680×gravity. Considering the spread of c_0 values for the different filaments, any treatment which decreases the structural viscosity (lowers the c_0 value) will cause an increase in the number of filaments with the chloroplasts displaced when centrifuged with an acceleration of 680×gravity. Likewise any treatment which increases the structural viscosity (raises the c_0 value) will cause a decrease in the number of filaments with the chloroplasts displaced.

Filaments of *Spirogyra* were immersed in tapwater solutions of 0.001*M* sodium glycocholate, 0.001*M* sodium taurocholate, 0.001*M* sodium oleate, and 0.0006*M* magnesium oleate. All solutions had a pH value of 7.5. After the filaments had been in the solution for the desired time, a group of them was removed, placed between strips of cotton soaked in the solution, and centrifuged. A group of untreated filaments (control) was centrifuged each time with the group of treated filaments. After a certain period of immersion, during which the relative viscosity had been determined at intervals for the experimental groups, the remaining filaments were transferred to tapwater and groups of them were centrifuged at intervals to note recovery from the effects of immersion in the solution. Following

centrifugation, the percentages of filaments having cells with the chloroplasts displaced were determined. For each experimental group between 350 and 450 filaments were examined.

Data

In recording the data, the percentage of untreated filaments with displaced chloroplasts was subtracted from the percentage of treated filaments with the chloroplasts displaced. (The two groups had been centrifuged synchronously.) Thus the figure recorded is the percentage of filaments in which the cells had the chloroplasts displaced as a result of the treatment.

TABLE 1

EFFECTS OF BILE SALTS UPON PROTOPLASMIC VISCOSITY

TIME OF IMMERSION (MINUTES)	PERCENTAGE FILAMENTS WITH CHLOROPLASTS DISPLACED	
	SODIUM TAUROCHOLATE	SODIUM GLYCOCHOLATE
5	7	0
20	26	5
40	35	23
60	47	28
90	30	34
Time in solution before removal to water (minutes)	100	70
Minutes in water:		
30	17	0
60	.....	0
90	0	.....

The results obtained for sodium taurocholate and sodium glycocholate are recorded in table 1. For the sodium taurocholate experiment an acceleration of 245×gravity was used for a period of 140 seconds; in the control groups the average percentage of filaments with the chloroplasts displaced was 14 per cent. For the sodium glycocholate an acceleration of 382×gravity was used for a period of 90 seconds; the average percentage of displacement in the control groups was 18 per cent. The taurocholate caused a somewhat greater decrease in structural viscosity than did the glycocholate: the maximum obtained for the former was 47 per cent as compared with a maximum of 34 per cent for the glycocholate. The glycocholate also required a longer time in which to bring about the decrease. In the taurocholate experiment the increase in structural viscosity after 90 minutes' immersion, as compared with the viscosity at 60 minutes, indicates a feature of its effect which is more exactly shown in the following oleate experiments (figs. 1–3).

In the oleate experiments the decrease in structural viscosity is not a cumulative effect but a fluctuating one: a decrease is followed by an increase which in turn is followed by another decrease, etc. The filaments recovered completely from the effect of the glycocholate 30 minutes after removal to tapwater and likewise were well on the way to a normal viscosity value after a 30-minute period in tapwater following treatment with the taurocholate.

The effect of sodium oleate during the first 6 minutes of immersion is recorded graphically in figure 2 (solid line); the effect during 135 minutes is recorded in figure 1. In the latter figure the solid line represents the effect of immersion in the oleate solution, and the broken line shows the trend of recovery when the filaments were removed to water after 135 minutes of immersion. The decrease in

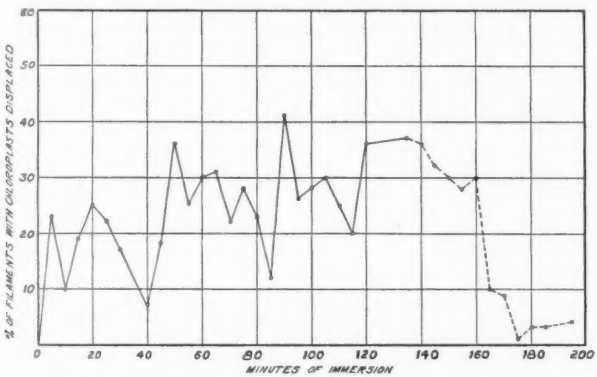
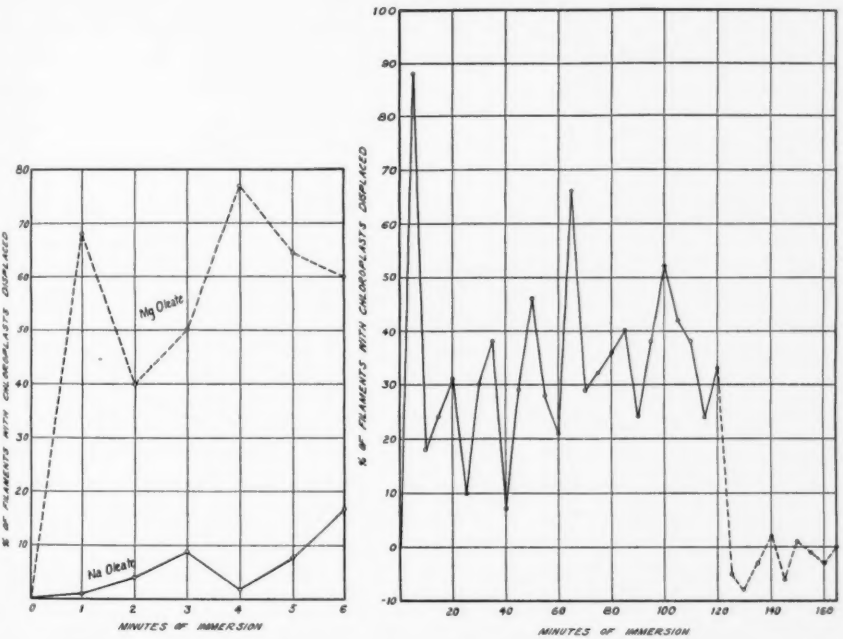


FIG. 1.—Effect of 0.001*M* sodium oleate solution upon structural viscosity of protoplasm in *Spirogyra* filaments as shown by centrifugation (solid line) and effect of removal to water (broken line).

structural viscosity was not pronounced during the first 6 minutes (maximum 17 per cent), and fluctuations were only slight. After a longer period of immersion (fig. 1) the fluctuations assumed major proportions and the decrease in structural viscosity became very significant (maximum 41 per cent). There was only partial recovery after 25 minutes in water, but after 30 minutes recovery was nearly complete. For this experiment the acceleration was 680×gravity applied for 50 seconds (excepting the seventh, eighth, and ninth recovery determinations for which it was 382×gravity applied for 90 seconds, and the tenth and eleventh for which it was 245×gravity applied for 140 seconds). The average percentage of chloroplast displacement in the control groups of filaments was 7 per cent for immersion determinations and 33 per cent for recovery determinations. The magnitude of the latter value is caused by the tendency of the c_0 value to change in response to variations in the intensity of illumination during such long periods of time as were employed in this and the following experiment.

The effect of the magnesium oleate is recorded graphically in figure 2 (broken line) and in figure 3. In the experiment for figure 3 the filaments were removed to water after 120 minutes' immersion. Although the magnesium oleate solution was only 0.0006*M* instead of 0.001*M*, and the acceleration was lower than that used in the sodium oleate experiment, the magnesium oleate had great initial effect (maxi-



FIGS. 2, 3.—Fig. 2 (left), effects of 0.001*M* sodium oleate solution (solid line) and 0.0006*M* magnesium oleate solution (broken line) upon structural viscosity of protoplasm in *Spirogyra* filaments as shown by centrifugation. Fig. 3 (right), effect of 0.0006*M* magnesium oleate solution upon structural viscosity of protoplasm in filaments as shown by centrifugation (solid line) and effect of removal to water (broken line).

mum 88 per cent), with pronounced fluctuations. These fluctuations also occurred during the longer period of immersion. The effect of the magnesium oleate in decreasing the structural viscosity did not exceed the effect of the sodium oleate as greatly in the longer period of immersion (maximum 66 per cent) as it did in the initial period. An important difference is also shown in the immediate recovery of the filaments from the effect of the magnesium oleate. When placed in water, the structural viscosity of the treated filaments within 5 minutes became slightly greater than that of the untreated (minimum −8 per cent). For this experiment the

acceleration was 382×gravity applied for 90 seconds in all determinations. The average percentage of chloroplast displacement in the control groups of filaments was 20 per cent for immersion determinations and 32 per cent for recovery determinations.

Discussion

The decrease in structural viscosity brought about by the bile salts and the oleates is interpreted as the result of a loosening of the protein cytoskeleton, probably through their effects upon linkages concerning fats and fatlike substances. This may occur in a way analogous to that which STEINHARDT (11) used to explain the dissociation of horse hemoglobin by amides. He proposed that binding forces between parallel polypeptide chains which arise between —N— and H —C— groups may be weakened or destroyed as a result of a similar association of O the introduced amides with the elements of the grid at the peptide bonds in competition with peptide bonds of adjacent chains. In a similar way substances such as the bile salts and oleates may weaken or destroy the homopolar cohesion bonds between lipophilic side chains described by FREY-WYSSLING through their association with the lipophilic side chains.

The possible effects of bile salts and oleates upon valence linkages—of the nature of those between lipids and proteins as proposed by OSBORNE (9), MÂCHEBOEUF (9), and SØRENSON (9)—would seem to be insufficient to account for the ultimate decrease in structural viscosity as observed.

ANSON (1) has found that detergents and bile salts denature ordinary proteins such as hemoglobin and egg albumin, and he considers that, since the detergents and bile salts differ widely in chemical composition but all have one property in common, their hydrophobic-hydrophilic polar character, it is this latter character upon which their reactions with proteins as well as their physiological reactions depend. BURKE (3) has found that the dissociation and denaturation processes in urea solutions of amandin and excelsin are closely correlated, if not one and the same process. The effects of the bile salts upon the protoplasmic proteins, as observed in this investigation, indicate that, although a dissociation occurs, it is not accompanied by denaturation, because there are fluctuations in structural viscosity and because the structural viscosity returns to the normal value when the filaments are transferred to water.

The fluctuations in structural viscosity are interpreted as the result of reassociations following the weakening or destruction of the linkages between the polypeptide chains brought about by the effects of the bile salts and oleates. These fluctuations in structural viscosity do occur, and their interpretation as one of dissociation and reassociation is compatible with the views of SØRENSON and others

(9) concerning soluble proteins. NORTHEN (8) has discussed such variation in protoplasmic viscosity.

Magnesium oleate has a much greater effect than has sodium oleate during the first 6 minutes of immersion (fig. 2). The difference in effect, although not so pronounced, continues during the longer period of immersion (figs. 1, 3). This difference might be explained as the result of a greater attraction between the fats and fatlike substances and the magnesium oleate. The fact that recovery from the magnesium compound's effect was immediate, however, whereas in the case of the sodium compound recovery occurred only after a period of time, makes such an explanation inadequate. The difference in effect could also be considered as a result of the control exercised by the cell membrane. The magnesium oleate would pass through a lipoidal membrane more rapidly than would sodium oleate, but it is doubtful whether its speed of entrance and departure would be great enough to account for the initial effect and the immediate recovery. It will be recalled that 68 per cent of the filaments had the chloroplasts displaced when centrifuged after 1 minute of immersion in the magnesium oleate solution and that 5 minutes after removal to tapwater the viscosity had increased to a value slightly higher than that of the untreated filaments.

The best explanation would seem to be based upon the distinction of "surface proteins" and the continuity between them and the other proteins of the cell. In the theory proposed by PETERS (6), a local change solely in the surface proteins (acceptors)—insufficient to cause permanent upset—could produce a decrease in protoplasmic viscosity by causing temporary dissolution of bonds which then "snap" back into place. The action of magnesium oleate and sodium oleate might then be postulated as consisting of two phases: (*a*) that concerned with the surface proteins; and (*b*) that concerned with the cytoplasmic proteins after the substance has entered the cell. The action upon the surface proteins would be of major importance during the initial period of immersion, and if the substance has a pronounced effect upon the surface proteins, the removal of the filaments to water would have an immediate consequence. During longer periods of immersion, the action upon the cytoplasmic proteins of the substance that has entered the cell would be of considerable importance. The action upon surface proteins and the action upon cytoplasmic proteins may then be additive in their effect. The major portion of the effect of the magnesium oleate would result from its action on the surface proteins, whereas the major portion of the effect of the sodium oleate would result from its action on the cytoplasmic proteins.

Summary

1. Groups of *Spirogyra* filaments were immersed in 0.001*M* tapwater solutions of sodium taurocholate, sodium glycocholate, and sodium oleate, and a 0.0006*M*

solution of magnesium oleate. At intervals, sample lots of filaments were centrifuged in their respective solutions. A control group of filaments from tapwater was always centrifuged with the group of treated filaments. An increase or decrease in the percentage of filaments having cells with the chloroplasts displaced indicated that the viscosity of the protoplasm had been decreased or increased, respectively.

2. All four substances decreased the structural viscosity. The decrease became more and more pronounced the longer the period of immersion in the solutions of the bile salts, except for an increase in structural viscosity at the end of 90 minutes' immersion in the sodium taurocholate solution. The oleates gave pronounced decreases in structural viscosity, followed by increases, which in turn were followed by decreases, etc. The effect of magnesium oleate was greater than that of sodium oleate.

3. As determined at 1-minute intervals during the first 6 minutes of immersion, the magnesium oleate produced a very large initial decrease in structural viscosity with major fluctuations, whereas the sodium oleate had a relatively small initial effect with only slight fluctuations.

4. After a period of immersion, the filaments were removed to tapwater and recoveries from the effects of the substances observed. The recovery from the effect of the sodium glycocholate was entirely completed, and the recovery from sodium taurocholate was half completed within 30 minutes. Recovery was completed within 5 minutes for filaments immersed in magnesium oleate solution but was not completed until 30 minutes in the case of sodium oleate.

5. The decrease in structural viscosity brought about by these substances may be the result of a weakening or destruction of bonds such as those existing between the lipophilic side chains of protein molecules. This is followed by reassociation, increasing the structural viscosity.

6. The rapidity and magnitude of the effect of the magnesium oleate, as compared with that of the sodium oleate, may be explained as the result of a greater effect of the former upon surface proteins and a restriction of the latter's effect more to the cytoplasmic proteins.

The writer expresses his gratitude to Dr. HENRY T. NORTHEN for his suggestions and assistance during the course of this investigation and acknowledges the assistance with laboratory details made possible by the N.Y.A.

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EFFECT OF INDOLEACETIC ACID ON THIN SECTIONS AND DETACHED SEGMENTS OF THE SECOND INTERNODE OF THE BEAN[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 518

J. M. BEAL

(WITH TWO FIGURES)

Introduction

The following experiments concern the effects of indoleacetic acid on detached segments and thin sections of the second internode of the Red Kidney bean (*Phaseolus vulgaris*) grown on WHITE'S nutrient solution. The nutrient solution was made up according to WHITE'S 1939 formula (2) and solidified to a soft gel by the addition of 0.5 per cent agar.

Methods and results

The experimental plants were grown in 6-inch pots containing garden soil and were kept under ordinary greenhouse conditions until ready for use. When the second internode had attained a length of 1–2 inches and the leaflets of the first compound leaf were beginning to expand, the stem was cut squarely across about 1 mm. below the base of the petiole of the compound leaf and immediately treated in one of the ways to be described, except for the one experiment in which the second internode was laterally treated.

SERIES 1.—After decapitation, a single application of 0.5 per cent indoleacetic acid in lanolin was spread evenly over the entire cut surface of the shoots. These were left on the plants and kept in ordinary daylight. Preliminary tests showed that the responses of treated stems were apparently as rapid and the histological changes were the same when an indoleacetic acid-lanolin mixture of this concentration was used as when a stronger mixture (such as 3 per cent) was applied, and that there was a marked reduction in injury to the cells at and near the surface of application with the weaker mixture. Only the weaker concentration was used, therefore, in the experiments involving the indoleacetic acid-lanolin mixture.

Following decapitation and application of the mixture, the same sequence of changes was observed in the treated stems in approximately the same time intervals as has been reported previously (1). Both gross aspects and histological responses parallel these descriptions very closely.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Transections 0.5 mm. or less thick were made from the apical swellings of stems, treated as described, 24 hours or more after treatment and were then placed on WHITE'S solidified nutrient. Before placing them on the nutrient, some were washed quickly in 95 per cent alcohol, some were dipped into a 1–1000 solution of $Hg(Cl)_2$ or a 2 per cent solution of $Ca(OCl)_2$ followed by rinsing in sterile distilled water, and some were placed immediately on the nutrient with no attempt at disinfection. There was little difference in the behavior of any of the segments, although there was somewhat greater fungus contamination when not sterilized.

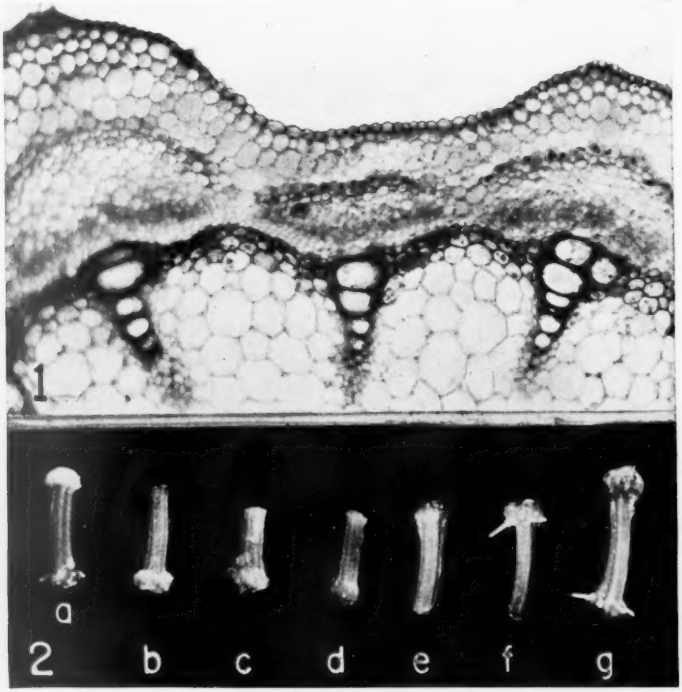
The responses of these sections were compared with those of comparable sections of second internodes treated with lanolin only and also with sections of freshly cut untreated stems which were also placed on the nutrient. In no case do the sections from the second internode not treated with indoleacetic acid proliferate noticeably, although they remain alive on the nutrient for several days. The sections from the swellings induced by the indoleacetic acid, made 24 hours or more after treatment, proliferate freely in the region outside the xylem cylinder, and in 2–4 days the proliferated tissues pile up to form a thick ruffled mass about the xylem. Roots frequently develop from the tissues outside the xylem cylinder and often attain a length of 5 mm. or more. The xylem ray tissues appear to be involved, but no proliferation of the pith was observed.

Pieces of tissue about 1 mm. square were taken—24 hours or more after treatment—from some of the stem swellings outside the xylem cylinder and placed on nutrient. These proliferate noticeably and often produce one or two roots each. It is probable that root primordia were present in some of the squares and that they continue development on the medium.

SERIES 2.—Other stems, without decapitation, were treated laterally by smearing the mixture in a narrow streak longitudinally along one side of the shoot. Within 1–1½ hours the treated stems begin to show negative curvature and by the end of 24 hours are markedly bent and slightly yellowed under the surface of application. At this time freehand sections treated with iodine show that almost all the starch in the endodermal cells beneath the surface of application has disappeared (fig. 1). The endodermal cells beneath the surface of application show radial elongation and an occasional division at the end of 30 hours. At 40 hours they have undergone active division and many show two to four derivatives. No starch can now be detected in these cells by the iodine test in freehand sections. Chloroplasts have also disappeared from cortical cells external to them. Root primordia become evident in the region of response at the end of 72–90 hours and may emerge at the end of 6–7 days. No changes are observable in the portion of the stem not covered by the mixture. The endodermal cells appear to contain

the normal amount of starch and show no signs of enlargement or of division, and the chloroplasts appear to be normal in the cortical cells outside them.

SERIES 3.—After decapitation and smearing as in series 1, pieces 2–15 mm. in length were immediately cut from the shoots and placed basal end down in petri



FIGS. 1, 2.—Fig. 1, transection from laterally treated second internode of bean 25 hours after smearing with lanolin mixture; note absence of starch in endodermal cells at left beneath surface of application and its presence at center and right where not treated. Fig. 2: *a* (6 days), apical smear with base on nutrient; both ends enlarged; *b* (6 days), basal end smeared, apical end on nutrient; base enlarged; *c* (6 days), basal end smeared and placed on nutrient; base enlarged; *d* (6 days), basal end on nutrient with 0.1 per cent aqueous indoleacetic added as thin film; basal end enlarged; *e* (6 days), apical end in nutrient with aqueous indoleacetic acid added; apical end enlarged; *f* (10 days), apical end on nutrient, basal end smeared; basal end enlarged and roots developed; *g* (7 days), apical end smeared and segment placed across gap on nutrient; both ends enlarged and base producing roots.

dishes containing a layer (about 2 mm. in thickness) of WHITE'S solidified nutrient. The shorter segments were placed basal end on the medium while those 6 mm. or longer had their bases submerged. The dishes were kept on a table near an east window, where they received light of moderate intensity.

At the beginning of the experiment some of the shoots, following decapitation and smearing, were left on the plants for periods of 2, 4, 8, and 12 hours before being cut and placed on the nutrient; but since these react in the same manner as those cut off and placed in the nutrient immediately after smearing, all the later experiments were carried out as first described for this series.

The treated segments all respond in essentially the same manner. Segments 2–5 mm. long show thickening throughout their entire length, with perhaps a slightly greater amount of initial swelling at the basal end. Ultimately the apical end enlarges to a greater extent than the basal. Sections of the shorter segments show the first noticeable cellular responses in the endodermis and cortex, followed by proliferation of the cells of the cambium, phloem parenchyma, and ray cells, as has been described (1) for treated stems left on the plants. About 72 hours following treatment, root primordia begin to develop at both the apical and basal ends and may emerge at 144 hours. Usually the roots are more numerous at the apical than at the basal end, but not always, and in most instances they are limited to a narrow circular zone extending about 1–2 mm. from either end of the segment.

The longer segments, particularly those more than 8 mm., elongate noticeably during the first 24 hours after being placed on the nutrient. Elongation continues for at least 48 hours, but less rapidly during the second 24-hour period. The total increase may be as much as 25 per cent of their length at the beginning of the experiment. These longer segments show noticeable basal and apical enlargement at the end of 48 hours. The enlargement becomes more marked at 72 hours, and roots may emerge at 144 hours after treatment (fig. 2*a*). As with the shorter segments, there may be a greater amount of initial swelling at the basal end; but after several days the apical swelling becomes greater owing to the development of more callus tissue at the apex. The central region of the segment between the apical and basal ends shows little or no evident change.

Control stem segments of similar lengths treated with pure lanolin, and some with no treatment other than cutting, were placed on the nutrient and left for similar periods of time. They elongate approximately as much as the treated segments but show no other detectable gross or histological changes during periods up to 6 days.

SERIES 4.—This duplicates series 3, except that the segments were placed on WHITE'S solidified nutrient, which was modified by omitting the sucrose. The segments remain green for 4–6 days but do not enlarge at either end nor show any detectable growth responses, other than elongation, after 96 hours.

SERIES 5.—This duplicates series 3 also, except that the segments were placed on WHITE'S solidified nutrient, which was modified by substituting calcium sulphate for calcium nitrate and omitting the potassium nitrate. As in series 4, no swelling was observable in the segments at 6 days.

SERIES 6.—Three methods of applying the indoleacetic acid were employed. In the first, following decapitation of the plants the stems were cut off in varying lengths and the morphological bases of the segments smeared with the lanolin mixture and placed apical end down on WHITE'S complete solidified nutrient. In the second the segments were smeared over their basal ends and then placed base down on the nutrient in such manner as to leave a ring of the lanolin around the base and possibly also some of the mixture on the cut surface of the base. In the third the segments were placed basal end down on the nutrient and then a thin layer of 0.1 per cent aqueous solution of indoleacetic acid was poured over the nutrient and in contact with the bases of all the segments.

The results are essentially the same for each of the treatments. The morphological base in each instance responds to the indoleacetic acid by yellowing and enlarging at about the same rate and in much the same manner as do decapitated and smeared stems when left on the plant. The morphological apex in none of these tests shows any evident changes (fig. 2*b*, *c*, *d*).

SERIES 7.—Segments of varying lengths were placed with the morphological apical end down on the nutrient and with the basal end up. A 0.1 per cent aqueous solution of indoleacetic acid was added in a thin film over the surface of the nutrient so as to come in contact with each segment. The apical ends of the segments enlarged, but the morphological base did not (fig. 2*e*). A modification of this experiment was performed by smearing the lanolin mixture over the cut surface of the apical ends of segments 5–15 mm. in length and placing the apical ends down in WHITE'S solidified nutrient so as to leave a ring of the mixture around the apical end. The apical end shows enlargement in the same time intervals as when left on the plants, but no evident changes take place in the basal ends (fig. 2*f*).

SERIES 8.—A strip of the solidified nutrient was removed across the entire center of certain of the culture dishes so as to prevent the possibility of diffusion between the two portions. Segments of second internodes were then treated in two ways: (*a*) the apical ends of pieces 13–20 mm. in length were smeared with the lanolin mixture and laid across the gap so that these ends rested on the nutrient at one side of the dish and the basal ends on the other side. Both apical and basal ends enlarge, as in series 3, and at the end of 6–7 days roots develop at both ends (fig. 2*g*). (*b*) Segments of similar lengths were treated basally and also laid on the nutrient. Only the basal portions enlarge, with no indication of apical enlargement after 7 days.

Discussion

It is evident from these experiments that the indoleacetic acid or some derivative from it moves almost entirely in a morphologically downward direction from the place of application. There is evidence from series 6, 7, and 8 that the acid or

its derivative does not move to any great extent in an acropetal direction. And for a response to occur, a certain concentration gradient of the indoleacetic acid or some derivative from it must be established downward from the surface of application. Associated with this gradient is a movement of food and nutrients upward in the internode, which enables the cells to grow, divide, differentiate, and mature. Among these substances are sugar and nitrogen needed in amounts greater than the quantities stored in the detached segments or sections.

As in the case of intact plants, when indoleacetic acid is applied to detached segments and thin sections in contact with WHITE'S nutrient there is a mobilization of sugar and nitrogenous substances to the place of application. When the latter are placed in contact with a complete nutrient with no indoleacetic acid present, there appears to be little change in the stored materials in segments or sections, little further growth, and practically no tendency toward formation of tumors and roots. If indoleacetic acid is applied there is digestion of the starch present, abundant growth and proliferation, and a direct absorption of foods and nutrients from the nutrient substrate. These are mobilized to the point or surface of application and used in the development of additional cells and tissues. When treated with indoleacetic acid the cellular changes and responses are similar, whether the stems are left on the plants or detached and placed in contact with the complete nutrient.

Summary

1. Detached segments and thin sections of the second internode of the bean, smeared apically with indoleacetic acid in lanolin and placed with the basal ends in contact with a complete nutrient, respond in essentially the same way as similarly treated stems on the plant. In addition to apical swelling and root formation, the detached segments enlarge and produce roots at the bases.

2. When treated basally and placed with the apical end on the nutrient, only the basal end enlarges. When treated apically and placed apical end on the nutrient, only the apical end enlarges.

3. Treated segments or sections placed on WHITE'S nutrient lacking either sugar or nitrogen show no enlargement.

4. Segments placed with either the apical or basal end on nutrient to which 0.1 per cent of aqueous indoleacetic was added enlarge only at the end in contact with the nutrient.

5. The movement of the indoleacetic acid, or some derivative from it, takes place almost entirely in a basipetal direction with little acropetal movement.

6. When indoleacetic acid is applied to detached segments or thin sections in contact with a complete nutrient, there is digestion of the starch present, abundant growth and proliferation, and a direct absorption of foods and nutrients which are

mobilized to the region of application and used in the development of additional cells and tissues. The cellular changes and responses in the detached segments and thin sections are similar to those which take place in intact treated second internodes.

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HISTOLOGICAL RESPONSES OF BEAN PLANTS TO TETRAHYDROFURFURYL BUTYRATE

WENDELL R. MULLISON

(WITH SEVEN FIGURES)

During the last several years a number of histological investigations have been made concerning the responses of various plants to growth-promoting substances (1, 2, 3). Tetrahydrofurfuryl butyrate when applied to bean plants as a lanolin mixture produces characteristic effects and was used in the study recorded here. The same technique has been followed as described in former studies of bean. A 2 per cent lanolin mixture of tetrahydrofurfuryl butyrate was applied terminally to the decapitated stem before the leaflets of the first compound leaf had expanded. Following the treatment, material[1] was collected daily for 3 weeks, the final collection being made within a month. Navashin's solution was employed as a fixative. The material was imbedded by the butyl alcohol paraffin technique and the sections cut at 12 μ.

Investigation

Gross effects

Bean plants show no gross effects until about 5 days after application of the mixture. The responses are somewhat similar to those caused by indoleactic acid, although the plants are slower to respond and the tumors are not so large. There is never more than a slight swelling of the stem immediately at or directly beneath the decapitated surface. As the tumor develops, a cavity forms in the central part of the stem; this is deepened by the upward growth of the callus. While growing upward, the callus also grows centrifugally, flaring outward over the edges of the cut surface, then beginning to grow downward, surrounding the top of the stem. At the same time centripetal growth is also taking place, resulting in complete closure at the top of the cavity, forming an irregular, flat-topped callus.

Histological effects

There is little noticeable effect until 3 days following treatment. The first sign of activity is the enlargement of some primary phloem cells, which shortly afterward become meristematic. Subsequently other tissues are involved.

There is no indication that the epidermal cells are stimulated. The paren-

[1] The plants used in this study were grown at the U.S. Horticultural Station, Beltsville, Maryland, during the spring of 1939 and made available for this study through Dr. E. J. Kraus.

FIGS. 1, 2.—Fig. 1 (left), 3 days after treatment: central part of pith disintegrated; initial activity in phloem. Fig. 2 (right), 7 days after treatment; great activity in pith and xylem, and some in phloem.

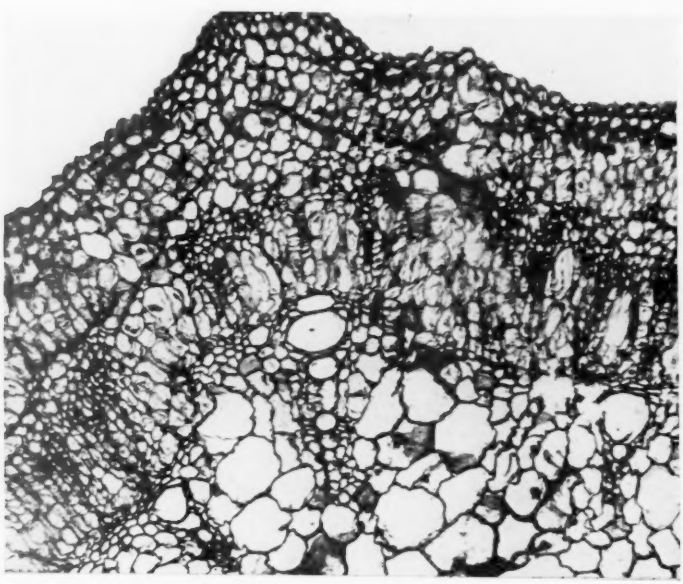


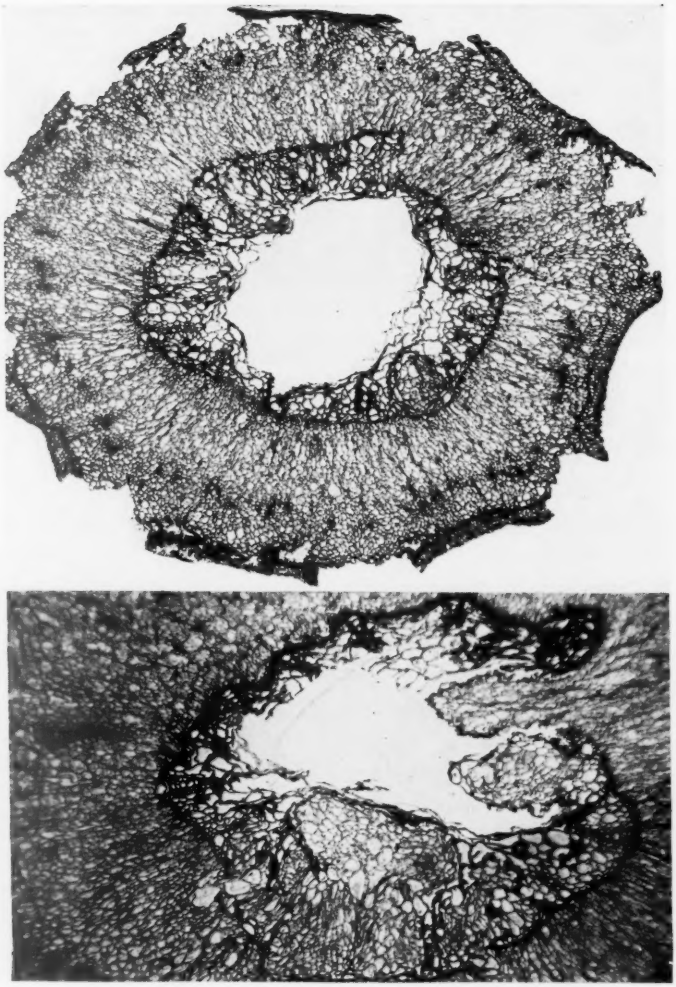
FIG. 3.—Four days after treatment: secondary xylem, phloem, and endodermis active, pericycle crushed; groups of greatly enlarged cells.

chymatous cells of the cortex also show practically no response. Later there is some sloughing of these tissues toward the tip of the stem owing to proliferation of tissues centripetal to them. The endodermis in young stages is not very active, although a few tangential divisions occur. In older stages the endodermis proliferates, giving rise to a narrow layer of parenchymatous cells. While the endodermis does not become very active, yet it responds to the stimulation at greater distances down the stem from the surface of application than do any of the other tissues. The cells of the pericycle are usually crushed or pushed from their original position by the growth of more centrally located tissues.

The phloem plays an active part in formation of the callus. Although the activity is initiated in the primary phloem, the secondary phloem and xylem become involved immediately, resulting in the formation of a wide band of meristematic tissue, most of which is derived from xylem. This band, together with meristematic areas initiated in the pith parenchyma, later forms the entire callus. The primary phloem is pushed outward, separating into small scattered units. One of the prominent features in this area in early stages is the occurrence of groups of three or four greatly enlarged cells, which are scattered at irregular intervals throughout the region. Cambial activity is slightly stimulated by the treatment, and somewhat more secondary xylem is found in the original stem portion than in untreated stems.

The xylem plays the most important role in callus formation. Delayed maturation of the metaxylem is often apparent. Sometimes cells that ordinarily would mature as metaxylem enlarge greatly and divide. At the tip of the stem the wide band of tissue derived chiefly from the secondary xylem and in small part from the phloem becomes extremely active and produces the largest part of the callus. In the callus tissue thus formed, highly disorganized vascular elements are differentiated, many of which are irregularly scattered groups of wound tracheids. Some of them, however, tie back to the vascular structure of the original stem. One characteristic feature in old callus tissue is the haphazard occurrence of groups of large cells.

Occasionally the ring of xylem about the stem is ruptured by the centripetal growth of large meristematic areas derived from the xylem parenchyma, which push aside and crush tissues in their path and penetrate into the central cavity. This cavity has appeared owing to disintegration of the pith. After the tumor has become prominent, the cavity tends to become filled with undifferentiated callus tissue, derived in part from meristematic areas arising in the parenchymatous cells of the pith adjacent to the primary xylem and in part from centripetally growing cells from the xylem as already described. The various stages in the development of the tumor are shown in the accompanying illustrations.



FIGS. 4, 5.—Fig. 4 (above), 7 days after treatment: epidermis and cortex being sloughed; scattered islands of primary phloem prominent near periphery; centripetal growth of xylem pushing through into pith cavity and meristematic areas developing in periphery of pith. Fig. 5 (below), 13 days after treatment: central cavity filling in by meristematic cells derived mainly from xylem and pith.

FIGS. 6, 7.—Fig. 6 (above), 30 days after treatment: central cavity completely filled across; vascularization of callus tissue and great amount of matured secondary xylem. Fig. 7 (below), 30 days after treatment: callus grown downward and enveloped top of stem; endodermis stimulated for considerable distance.

Discussion

The reactions of bean plants to the several growth-promoting substances which have already been reported are sufficiently different so that in most cases they can be told apart by their gross aspects. Tetrahydrofurfuryl butyrate causes a slightly irregular, flat-topped tumor which grows downward, enveloping the stem below it for a short distance; the stem itself swells but slightly, and no adventitious roots are produced. Stems treated with naphthaleneacetic acid and alpha naphthalene acetamide also remain flat topped, but they differ greatly in amount of reaction. The former swell so much that a definite shoulder is formed about 1 cm. below the point of application, and the swelling may be apparent as far as 7 cm. below the shoulder. On the other hand, there is very little proliferation of any tissue treated with alpha napthalene acetamide; the stem becomes very much hardened, and only a few roots are produced at the point of application.

Indoleacetic and indolebutyric acids produce large apical tumors which push up irregular warty masses overtopping a definite crown of adventitious roots; the stem becomes very swollen. The swelling of the stem caused by indolebutyric acid is the more marked of the two, extending much farther downward, and adventitious roots may be seen not only at the original point of application but 5–10 mm. below as well.

There are also marked differences in reaction of certain tissues to these five growth-promoting compounds. In all cases there is little or no proliferation in the epidermis and pericycle, although the cell walls of the latter are thickened by alpha naphthalene acetamide.

Reactions of the cortical cells range from no activity through marked proliferation. Tetrahydrofurfuryl butyrate causes no reaction; indoleacetic acid causes simple cell enlargement; indolebutyric acid causes marked enlargement; alpha naphthalene acetamide causes thickening of the cell walls; and naphthaleneacetic acid causes great activity.

After applications of tetrahydrofurfuryl butyrate there is moderate activity of the endodermis, with no formation of vascular tissues or root primordia. With all other compounds the endodermis shows great activity, vascular bundles becoming differentiated in its derivatives. In the case of indoleacetic acid the endodermal derivatives give rise to some of the root primordia, while in the case of indolebutyric and naphthaleneacetic acid it is involved along with other tissues in root formation.

When treated with tetrahydrofurfuryl butyrate the phloem becomes a fairly active tissue, producing part of the callus and containing some highly disorganized groups of tracheids. Alpha naphthalene acetamide causes but little activity in the phloem. The other three compounds cause great activity there, producing a mass of tissue with many vascular strands running through it.

Tetrahydrofurfuryl butyrate slightly stimulates cambial development and causes it to produce a little more secondary xylem than in the untreated stem. The other four cause pronounced proliferation. In these latter cases differentiation of cambial derivatives is delayed, except in the case of alpha naphthalene acetamide, where the production of considerable secondary xylem is brought about

TABLE 1

HISTOLOGICAL EFFECTS OF GROWTH-PROMOTING COMPOUNDS ON BEAN

Tissue	Response		
	Tetrahydrofurfuryl butyrate	Indoleacetic acid*	Alpha naphthalene acetamide
Epidermis	No activity	Little activity	No activity
Cortex	No activity	Disappearance of chloroplasts and starch grains; some cells enlarged	Thickening of cell walls
Endodermis	Slight activity but no differentiation of vascular bundles	Great activity; differentiation of vascular bundles	Moderate activity; differentiation of vascular bundles
Pericycle	Little activity	Little activity	Fibers much thickened
Phloem	Activity; produces part of callus	Great activity; differentiation of vascular bundles	Little activity
Cambium	Stimulated; produces more secondary xylem than in untreated plants but not nearly so much as alpha naphthalene acetamide	Divides rapidly but differentiation of derivatives delayed	Most active tissue; much secondary xylem
Xylem	Most active tissue; produces most of callus	Little activity	Little activity; increased thickness of cell walls
Pith rays	Very active but no differentiation of vascular bundles	Very active; differentiation of vascular bundles	Activity
Pith	Cells adjacent to primary xylem active	Eventually very active and produces most of callus	Slower than indoleacetic and not so active; differentiates a few vascular bundles
Tissues involved in root formation	None	Pith rays and phloem† Endodermis	Pith rays

* Indolebutyric and naphthaleneacetic acids produce about the same response as indoleacetic acid.
† Most of the roots are derived from these tissues.

by the cambium, the most active tissue. Tetrahydrofurfuryl butyrate causes the cambial derivatives that would mature as xylem to become meristematic and to play an extensive role in callus formation, while in the other three the xylem plays only a slight role.

In all cases the pith rays become highly meristematic. Indoleacetic and indolebutyric acids cause differentiation of many vascular bundles in the pith rays.

The others produce none. Tetrahydrofurfuryl butyrate kills the cells of the central portion of the pith, but later the parenchyma of the pith adjacent to the primary xylem becomes very active and in conjunction with the xylem fills up the cavity with parenchymatous cells. Naphthaleneacetic acid causes little activity in the pith at any time. In the other three cases the pith is slow to respond, but eventually becomes quite active and produces a mass of callus in which vascular bundles become differentiated.

Roots are never produced at the tumor or near it after application of tetrahydrofurfuryl butyrate. In the other four cases root primordia are produced. Alpha naphthalene acetamide initiates root production solely in the pith rays. Naphthaleneacetic acid also stimulates the pith rays to produce root primordia, and in addition it causes their occasional formation in the endodermis. Indoleacetic acid causes root production mostly in the pith rays and phloem, and also to some extent in the endodermis, while indolebutyric acid produces roots in which the endodermis and phloem are both involved; it may also form roots in the pith rays.

The similarities and differences in the histological behavior pattern of bean to the growth-promoting compounds thus far investigated are summarized in table 1.

Summary

There are striking differences between the reaction of bean to tetrahydrofurfuryl butyrate and the other substances thus far investigated. The most prominent is the complete absence of root primordia arising either as a part of the tumor or near it. The xylem is the most active tissue, which with its derivatives forms by far the largest part of the callus, although the phloem is also involved. The callus, while having differentiated a considerable number of wound tracheids, some of which tie back with the vascular tissue of the stem, is largely composed of parenchymatous cells, scattered among which are groups of extremely large cells and areas of meristematic activity. The cavity formed by disintegration of the central region of the pith tends to be filled in with parenchymatous cells derived in part from the pith and in part from the xylem. Cambial activity is stimulated and produces a little more secondary xylem than in untreated stems. There is a moderate amount of activity in the endodermis, with no differentiation of vascular tissue. The endodermis, however, responds to the stimulation of tetrahydrofurfuryl butyrate farther down the stem from the surfaces of application than does any other tissue. Another striking difference is the localization of the response within 1–3 mm. of the site of application.

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NITROGEN CONTENT OF SOUND AND DECAYED CONIFEROUS WOODS AND ITS RELATION TO LOSS IN WEIGHT DURING DECAY

R. E. HUNGATE

Nitrogen changes during wood decay are of interest not only as they affect forest soil fertility but also from the standpoint of the physiology of the fungi concerned. The amount of nitrogen in wood is extremely small. In the present studies it has been found in *Pinus monticola* to average 0.048 per cent of the dry weight of the sapwood and 0.031 per cent of the heartwood. In spite of such low contents, many investigations (**2, 7, 12**) show that pine wood may lose more than one-third of its weight during attack by pure cultures of wood-destroying fungi. If the nitrogen content of the woods used was about the same as that found in the woods of the present report (FINDLAY (**2**) gives a value of 0.04 per cent), it is evident that the quantity of carbon compounds decomposed is almost 700 times the amount of nitrogen utilized, unless unknown additional nitrogen sources were available to the fungi. This is very much larger than the ratio of 30 to 1 reported for other cellulose-destroying fungi (**3, 6**). The results of FINDLAY (**2**) and SCHMITZ and KAUFERT (**7**) indicate that addition of a suitable nitrogen source to the wood increases the rate of decay in laboratory cultures. It has seemed of interest to investigate the natural destruction of wood by fungi and to determine whether the nitrogen in the wood is the sole source of this necessary element.

Material and methods

Samples of sound and decayed wood of several species of coniferous trees were collected and used for determinations of dry weight, specific gravity, and nitrogen content. Data on specific gravity were obtained in order to allow for any increase in percentage of nitrogen due to a decrease in other materials. Since the specific gravity is known, it is possible to compare the nitrogen content per volume of sound wood with an equal volume of decayed wood.

There are changes in the volume of wood during decay (**5**), particularly during the later stages, so that comparison on the basis of equal volumes may introduce an error. In obtaining samples of decayed wood for the present analyses, only those which seemed to have retained their original shape and were without large cracks and fissures were selected. This not only reduced errors due to volume changes but also assured that the samples remained intact during the determinations of specific gravity. Although no data on volume change during decay were

obtained, any change would probably be in the direction of decreased volume and therefore would give higher values for the percentage of nitrogen in a unit volume of decayed wood than for the same volume of sound wood, provided there was no change in absolute amount.

The woods were from virgin, over-mature forests on the slopes adjacent to the east shore of Priest Lake, Idaho, in the vicinity of Lion Head Bay. Samples of sound and rotten western white pine (*Pinus monticola*), western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*), and white fir (*Abies grandis*) were obtained. An ax or saw was used to cut out a portion of the selected log to give a sample about 6–8 inches long (with the grain) and containing both sapwood and heartwood. Care was taken to avoid inclusion of insects or their borings in the sample. When possible, the wood was cut from the rotten logs at a distance of 6–10 feet from the point of contact with the ground. This precaution decreased the possibility of nitrogen absorption from the soil by such physical methods as capillarity. It was also desired to investigate natural wood decomposition under conditions where additional nitrogen was not readily available, in order to determine the magnitude of weight loss under these circumstances. If soil nitrogen was used for the decay of the wood thus selected, it must have been transported for a considerable distance.

Samples were immediately dried in the warming pan of a kitchen stove and after shipment to Austin, Texas, were dried overnight at a temperature of 106° C. They were allowed to remain in air for some time, were weighed, and their specific gravity determined by weighing the amount of ethyl alcohol (s.g. 0.80) which they displaced when immersed in a bucket full of alcohol, provided with an overflow spout. Alcohol was selected because of its low surface tension and because it speedily penetrated the wood. Before immersing the sample in the test bucket, it was placed in another bucket of alcohol and allowed to stand until it was saturated. This prevented a false value due to uptake of liquid by the unwetted wood. Transfer from the preliminary to the test alcohol was accomplished quickly so that no alcohol was lost from the interior of the sample. The displaced alcohol was collected and weighed quickly so that the error due to evaporation could be neglected. A determination on a sample using alcohol was checked with mercury and similar results obtained. The alcohol was analyzed for its nitrogen content before and after use and it was found to be negligible in both cases. Thus no error was introduced owing to nitrogen added or removed by the alcohol.

After the specific gravity determination, the sample was again dried at 106° C. and weighed. There was no significant change from the first weight. It was ground in a Wiley mill until it would pass a 1-mm. sieve. The ground wood was collected in paper bags and set aside for the determinations. Thus the nitrogen analyses were made after the wood had come to more or less of an equilibrium with the air.

Humidity changes would cause some variations in the weight of the sawdust (13), but this error was estimated to be less than the error of 4 per cent in determining nitrogen.

The low percentage of nitrogen in wood makes it difficult to determine the total nitrogen accurately, since a large sample must be used in order to get sufficient nitrogen to measure. The use of micro-methods does not solve the problem, since these methods are adapted to minute amounts of materials containing fairly large percentages of nitrogen but not to material containing minute percentages.

The Dumas, Kjeldahl, and ter Meulen methods for total nitrogen have been considered during the course of the present work. The Kjeldahl has given the most reproducible results. In the earlier analyses the ter Meulen methods were used (8, 9, 10) and occasionally gave (with duplicate samples) values which differed by as much as 25 per cent. The several modifications of the ter Meulen technique were tried but none was particularly satisfactory. The disagreement between duplicate samples was considerably greater than the error in titration.

Dr. A. J. HAAGEN-SMIT at the California Institute of Technology kindly investigated the feasibility of determining nitrogen by the Dumas method when it is present in such small percentages and found that the errors arising from the use of the necessarily large sample are so great as to make the method unsuitable. In view of the superiority of the Dumas method for total nitrogen, it is unfortunate that it has not been possible to check the Kjeldahl results against it. The values obtained by the ter Meulen and the Kjeldahl methods are of the same magnitude, however, and it is probable that the found nitrogen values are not far from the true values.

The usual macro-Kjeldahl analysis was found to involve errors owing to the large amount of fluid in which the final titration was accomplished. It was also time-consuming. After a few experiments it was decided that best results could be obtained by digesting samples of semi-micro size (200 mg.) and distilling and titrating on a micro-scale. The 200-mg. sample was placed in a 100-cc. Kjeldahl digestion flask with 1 gm. of potassium sulphate, 0.1 gm. mercury, and 3 cc. of concentrated sulphuric acid. Digestion was completed in 20–30 minutes; and after cooling, the digest was diluted with 10 cc. of cold water.

A Pregl micro-still was constructed, similar to the usual apparatus, except that a bent tube to serve as an additional trap for sodium hydroxide droplets was inserted in the main flask, so that the vapors passed through it before entering the customary trap. This arrangement was found to be so effective that rapid steam distillation for 5 minutes through an ammonia-free strongly alkaline solution resulted in driving over basic materials equivalent to less than 0.02 cc. of 0.006 normal acid. The distillation was carried out as usual after addition of 10 cc. of the NaOH-Na_2S mixture, except that the ammonia driven off was titrated as it

TABLE 1

NITROGEN CONTENT AND SPECIFIC GRAVITY OF WOOD SAMPLES

Wood and sample no.		Sound				Sample no.	Rotten			
		Sapwood		Heartwood			Sapwood		Heartwood	
		Mg. N. / cc.	Spec. grav.	Mg. N. / cc.	Spec. grav.		Mg. N. / cc.	Spec. grav.	Mg. N. / cc.	Spec. grav.
Pine	1	0.17	0.419	0.12	0.434	9	0.22	0.329	0.17	0.443
	3	0.23	0.497	0.18	0.506	10	0.19	0.426	0.14	0.425
	18	0.32	0.501	0.22	0.548	13	.....	.....	0.18	0.394
	37	0.21	0.441	0.14	0.526	14	0.24	0.343	0.15	0.387
	38	0.30	0.536	0.21	0.551	19	0.17	0.303	0.11	0.420
	39	0.22	0.497	0.15	0.561	22	0.17	0.344	0.13	0.442
	40	0.26	0.541	0.14	0.543	30	0.20	0.298	0.15	0.488
	42	0.19	0.465	0.11	0.460	32	0.14	0.219	0.10	0.357
	48	0.18	0.380	0.15	0.493	33	0.16	0.346	0.12	0.370
	51	0.18	0.426	0.14	0.428	44	0.29	0.480	0.20	0.412
	.....	.....	.....	.....	.....	49	0.26	0.375	0.17	0.355
	.....	.....	.....	.....	.....	50	0.18	0.424	0.16	0.486
Hemlock	2	0.24	0.495	0.17	0.504	12	0.19	0.328	0.19	0.436
	11	0.23	0.492	0.17	0.494	20	0.17	0.413	0.14	0.397
	16	0.27	0.485	0.20	0.505	21	0.16	0.292	0.15	0.288
	17	0.22	0.424	0.23	0.471	25	0.17	0.385	0.18	0.388
	24	0.19	0.564	0.17	0.564	26	0.23	0.382	0.16	0.428
	27	0.21	0.486	0.18	0.479	28	0.23	0.332	0.18	0.341
	29	0.20	0.500	0.16	0.521	34	0.25	0.392	0.20	0.407
	35	0.21	0.530	0.19	0.481	41	0.15	0.331	0.24	0.329
	.....	.....	.....	.....	.....	43	0.27	0.439	0.22	0.423
	.....	.....	.....	.....	.....	45	0.26	0.376	0.18	0.404
	.....	.....	.....	.....	.....	46	0.29	0.344	0.22	0.406
	.....	.....	.....	.....	.....	47	0.32	0.452	0.16	0.471
Cedar	5	0.18	0.370	0.18	0.401	7	0.33	0.300	0.20	0.424(s)*
	6	0.18	0.381	0.19	0.384	8	0.15	0.275	0.14	0.319(s)
	15	0.20	0.316	0.19	0.410	52	0.37	0.378	0.21	0.419(ps)
	23	0.24	0.382	0.16	0.402	55	0.25	0.264	0.19	0.334(ps)
	57	0.24	0.290	0.19	0.324	56	0.27	0.305	0.17	0.357(ps)
	62	0.19	0.394	0.17	0.444	61	0.16	0.331	0.16	0.338(ps)
	65	0.22	0.312	0.16	0.388	63	0.20	0.346	0.15	0.289(ps)
	68	0.16	0.328	0.12	0.364	64	0.16	0.301	0.15	0.305
	70	0.24	0.392	0.13	0.395	69	0.22	0.354	0.13	0.353(ps)
	71	0.37	0.433	0.23	0.470	.....	.....	.....	.....	.....
	74	0.30	0.375	0.18	0.433	.....	.....	.....	.....	.....
Fir	60	0.43	0.411	0.14	0.377	66	0.17	0.204	0.12	0.378
	73	0.16	0.492	0.13	0.393	72	0.26	0.364	0.24	0.402
	77	0.38	0.497	0.17	0.511	75	0.17	0.296	0.26	0.280
	79	0.17	0.354	0.14	0.347	76	0.43	0.306	0.31	0.479
	80	0.34	0.504	0.18	0.519	78	0.24	0.242	0.18	0.260

* (s) sound; (ps) partially sound.

came over instead of being trapped in an excess of acid and back titrated. With this modification it was possible to keep the volume of liquid in the titration vessel down to less than 10 cc., and the accuracy of the titration was accordingly increased. This particular change was taken over from the ter Meulen method, and it not only increased the accuracy but also was quicker; the point at which no more ammonia came over could be accurately detected and the distillation stopped at that point, instead of continued over for an additional margin of safety. Initially some difficulty may be experienced in catching the first rush of ammonia without letting the titration vessel become too alkaline, but as soon as the approximate amount of nitrogen in the sample is known it is easy to maintain a suitable excess during the critical period.

Nitrogen determinations were always run in duplicate, and with few exceptions the duplicates agreed to within 4 per cent of the total nitrogen when the latter was 0.05 per cent. This absolute error of 4 gammas of nitrogen is about equal to one drop of the acid used. The agreement was achieved not only by the writer but after a few trials also by students with little previous training. With each set of nitrogen determinations a blank was run on the reagents and a correction made. Further refinements probably would have increased the accuracy, but it was not thought necessary for the present studies, using averages of many samples.

Analytical results

Table 1 gives the results of the determinations. Each nitrogen value is the average of duplicate determinations.

The mean nitrogen content and the probable error of the mean for each species in table 1 have been calculated and are assembled in table 2.

The results obtained with pine, hemlock, and cedar show no significant change in the total nitrogen content during decay. The rotten sapwood of the fir shows less nitrogen than the sound sapwood, but the error here is larger owing to the smaller number of samples and the greater variation. The figures for fir heartwood show an increase in nitrogen content with decay. The difference is not large, however, and coupled with the fact that the rotten sapwood shows a decrease in nitrogen, the evidence for a total increase is not convincing.

In general the analytical results indicate that there is no significant change in nitrogen during decay. This agreement between the nitrogen values for sound and decayed wood also suggests that the decayed samples had not undergone any significant shrinkage in volume. It is true that a similarity in the nitrogen content between sound and decayed wood might result if the loss in nitrogen during decay exactly balanced any gain. Unless the loss and gain were linked in some way, however, it does not seem probable that they would be exactly equal. The close agreement between the nitrogen content of sound and rotten wood in these analyses is

better interpreted as nitrogen retention. If this is correct, the nitrogen used by the fungi attacking the wood cannot be greater than the total nitrogen content.

From the mean values for the specific gravity of the sound and decayed samples, the amount of materials (principally non-nitrogenous) disappearing during decay can be calculated. The average specific gravities are given in table 3. The difference column gives the average grams of wood per cubic centimeter that dis-

TABLE 2

SUMMARY OF DATA ON NITROGEN CONTENT
AVERAGE NITROGEN IN MG./CC.

Wood	Sapwood		Heartwood	
	Sound	Rotten	Sound	Rotten
Pine	0.226±0.010	0.202±0.009	0.156±0.007	0.148±0.006
Hemlock	0.221±0.006	0.224±0.010	0.184±0.005	0.185±0.006
Cedar	0.229±0.012	0.234±0.017	0.173±0.006	0.167±0.006
Fir	0.296±0.033	0.254±0.027	0.152±0.006	0.222±0.020

TABLE 3

AVERAGE SPECIFIC GRAVITIES OF WOOD SAMPLES IN GM./CC.

Wood	Sapwood			Heartwood		
	Sound	Rotten	Difference	Sound	Rotten	Difference
Pine	0.470	0.353	0.117	0.505	0.415	0.090
Hemlock	0.497	0.372	0.125	0.503	0.393	0.110
Cedar	0.361	0.317	0.044	0.401	0.349	0.052
Fir	0.452	0.282	0.170	0.429	0.360	0.069

appeared during decay. This can be compared with the nitrogen per cubic centimeter of sound wood, as has been done in table 4.

The values of the ratio for pine, hemlock, and fir are rather similar, whereas for cedar lower values are found. These low values can be ascribed in part to certain difficulties in securing samples of the wood of this species. The central heartwood is not resistant to decay and in standing and fallen logs is usually rather rotten. The fungus attack in this central portion results in such checking and shrinking that comparisons on the basis of volume are impossible, thus preventing use of these well-decayed portions. The outer heartwood is extremely resistant, and in some cases logs which have been down for as long as 100 years show little sign of extensive decay (5). When decay does appear it is usually not uniform but is con-

fined to narrow strips between areas of sound wood. In collecting the samples no attempt was made to separate the rotten from the sound portions, since it was thought that the difference in fungus attack might be due to differences in the nitrogen in local areas of the wood, and thus a selection might give high or low nitrogen values which should not be compared with the average nitrogen values determined for sound wood. Table 3 shows that the weight of cedar heartwood decomposed is considerably less than for the other species. Since only part of the wood had decomposed, and yet the nitrogen in the sound part has been included in the carbohydrate/nitrogen ratio, the value of the ratio would be expected to be low. The opposite difficulty was experienced with cedar sapwood, in that it decays very quickly and some of the samples of down logs classified as sound and containing sound heartwood had the sapwood decayed. Thus the calculated weight of wood disappearing during decay is lower than the real loss and the carbohydrate/nitrogen ratio is correspondingly reduced.

TABLE 4

RATIO BETWEEN COMPOUNDS DISSIMILATED AND TOTAL NITROGEN

Wood	Sapwood			Heartwood		
	Wood disappearing (mg.)	N/cc. (mg.)	Ratio	Wood disappearing (mg.)	N/cc. (mg.)	Ratio
Pine	117	0.226	518	90	0.156	576
Hemlock	125	0.221	565	110	0.184	597
Cedar	44	0.229	192	52	0.173	300
Fir	170	0.296	575	69	0.152	454

The ratios for fir are less reliable than those for pine and hemlock because of the fewer samples analyzed and their greater variation. The increase in nitrogen during decay in the heartwood and the decrease in the sapwood suggest the possibility that nitrogen was transferred from sapwood to heartwood. A conduction of nitrogen for a distance of about 9 inches from soil through wood has been detected in a laboratory culture of termites on *Pinus radiata* in which the wood was attacked by fungi. It is conceivable that a similar transfer might have occurred across the fir logs from the sapwood to the heartwood. If so, then the amount of nitrogen used during the decay of either sapwood or heartwood cannot be accurately ascertained with the available data, and the carbohydrate/nitrogen ratio is correspondingly less reliable. The values for fir, however, are of the same general magnitude as for pine and hemlock.

Discussion

One of the numerous factors affecting the relative nitrogen content of sound and decayed wood is the amount of nitrogen transferred into the fruiting bodies of the fungi. The sporophores contain a larger percentage of nitrogen than is found in the wood, and if there are no additional sources the nitrogen content of the wood might be expected to be low when fruiting bodies have been formed. If, however, the nitrogen content of the food of fungi is low, the nitrogen content of the fungus body is reduced (**11**). FINDLAY (**2**) gives a nitrogen content of 0.68 per cent for the mycelium of *Polystictus versicolor* growing on wood. If the nitrogen in the fruiting bodies on wood were about this same percentage, it would take 14 gm. of wood with a nitrogen content of 0.05 per cent to contain sufficient nitrogen for 1 gm. of fungus. With an error of 4 per cent in determining nitrogen, a draining of nitrogen from the wood by 1 gm. of fungus would not be detected if the area for nitrogen collection included more than 350 gm. In all the logs selected for the present analyses the weight of the wood was estimated to be much more than 350 times the weight of the sporophores. Thus any accumulation of nitrogen in the fruiting bodies would not be detected in these analyses. Two samples of rotten hemlock showed conspicuous accumulations of mycelium at many spots. One of these, no. 46, contained a higher than average amount of nitrogen per cubic centimeter, 0.29 per cent, but the other, no. 20, was lower than average, 0.17 per cent.

The fact that prominent fruiting bodies are often formed demonstrates a capacity on the part of the fungi to transport nitrogenous materials for some distance through the wood. Since decaying logs are often partially imbedded in the ground it is possible that soil nitrogen may also be transported. Common observation indicates that the portion of the log immediately in contact with the ground is usually the most decayed. It is probable that nitrogen as well as moisture conditions is concerned, and as just stated, this has been observed in a laboratory culture. The similarity between the nitrogen content of sound and decayed logs in the present analyses indicates that soil nitrogen was not transferred as far as the points from which samples were taken, at least not in detectable amounts. Since precautions were taken to obtain rotten samples at a considerable distance from ground contacts, a transfer of nitrogen into wood in close proximity to the soil would not have been detected.

The experiments of FINDLAY (**2**) and of SCHMITZ and KAUFERT (**7**) bear directly on the importance for decay of the nitrogen in wood. FINDLAY compared the loss in weight of Sitka spruce with and without added nitrogen when attacked by *Trametes serialis*. Addition of 0.5 per cent ammonium nitrate gave a loss of 15.08 per cent, whereas 10.62 per cent was dissimilated in the control without added nitrogen. One per cent peptone increased the loss from 25.83 to 40.82 per cent.

The amounts of added nitrogen based on the weight of wood were 0.175 per cent for the ammonium nitrate and 0.167 per cent for the peptone. The nitrogen content of the wood was 0.04 per cent. SCHMITZ and KAUFERT found similar effects. Addition of asparagine, in which the nitrogen amounted to about 0.04 per cent of the weight of the Norway pine sapwood, increased the weight loss to 35.5 per cent when the controls without added nitrogen showed a loss of 33 per cent. With 0.2 per cent added nitrogen as asparagine the loss was 46.7 per cent; with 0.4 per cent the loss was 47.5 per cent. Ammonium nitrate in which the nitrogen amounted to 0.066 per cent of the weight of the wood increased the loss to 37.8 per cent, but higher concentration decreased the rate of decay. The naturally occurring nitrogen in the wood was not determined but probably was about 0.04–0.05 per cent.

The experiments with added nitrogen suggest that growth of the fungi of wood decay is limited by the amount of available nitrogen in the wood. They also emphasize the extreme economy with which the nitrogen in the wood is utilized. Additional nitrogen equal in amount to that already present increased the weight loss only one-tenth, and addition of more than four times the amount increased it only one-half. From these figures it can hardly be concluded that the fungi are in a critical state of nitrogen starvation.

Higher values for the carbohydrate/nitrogen ratio might have been observed in the present analyses had it been possible to work with samples that were more completely decayed. The data on specific gravity for pine and hemlock indicate that about 25 per cent of the wood had disappeared. The data of others indicate that as much as 33 per cent may disappear in a pure culture. If it were possible to get accurate specific gravity determinations on samples which were more completely decayed, the values of the ratio of carbohydrate to nitrogen would probably be larger than those calculated from the present analyses.

Another factor which might have influenced the value of the carbohydrate/nitrogen ratio was the method of selection of the sound wood samples. Since it was preferred not to disfigure the living trees, most of the samples were from down logs. Standing or recently fallen dead trees were selected; and if after cutting into the log the wood appeared to have the color, texture, and strength characteristic of sound wood, it was accepted as suitable. Since the initial stages of fungus decay are not always macroscopically evident (4), it is possible that some of the sound samples had undergone a slight loss of weight due to fungus attack. This would make the carbohydrate/nitrogen ratio somewhat lower than if completely undecayed wood were used. Where samples of both living and fallen dead trees were collected there was no indication that the fallen ones had a significantly lower specific gravity, except in the case of the cedar sapwood already mentioned. Since it is improbable that the initial mycelium of infection would be more effective in bringing nitrogen into the wood than the later mycelium, these questions as to the

soundness of the sound samples do not affect the conclusion that the amount of nitrogen in the wood is unchanged during decay.

It should be emphasized that the calculated carbohydrate/nitrogen ratios are not the ratio between the carbohydrate utilized and the nitrogen utilized, but are rather the ratio between the carbohydrate disappearing as loss in weight and the total nitrogen present. If, as the work of BRAY and ANDREWS (1) indicates, some of the carbon compounds in the wood are attacked and changed by the fungi without necessarily being decomposed to carbon dioxide and water, then the ratio between carbon compounds used by the fungi to total nitrogen in the wood will be larger. Similarly, if some of the nitrogen in the wood is not used by the fungi, then the ratio between carbon compounds used and nitrogen used would be still larger. All these considerations point to the fact that the ratios calculated are conservative estimates of the economy with which wood-destroying fungi are able to use nitrogen in the destruction of the carbohydrate materials.

The high carbohydrate/nitrogen ratio might be interpreted as indicating a cycle of nitrogen in the wood, in which this material is used over and over by various organisms. Apparently, however, a cycle in which numerous organisms are concerned is not necessarily a factor in the observed nitrogen efficiency, since pure cultures of fungi are able to decompose as much wood as was calculated from these analyses to have disappeared during natural decay. If there is repeated use of nitrogen, the repetition is accomplished by the same organism. It is interesting to speculate on whether such a physiological mechanism may explain the nitrogen economy observed.

Although apparently unable to fix atmospheric nitrogen or to transport this material over very great distances from soil into wood, although living in a medium containing occasionally as little as 0.25 mg. of nitrogen per gram of other materials, yet the wood-destroying fungi are able to penetrate and spread, dissimilating relatively huge quantities of the non-nitrogenous materials, and ultimately forming prominent fruiting bodies. The problems presented in the decomposition of a resistant carbohydrate material poor in nitrogen have apparently been met during the course of evolution of this group of fungi, and this may account in part for their unique position as the prime agents of wood decay.

Summary

1. A satisfactory method for the determination of total nitrogen in wood is described.

2. Determinations of specific gravity and nitrogen content for sound and decayed woods indicate that there is little change in the total nitrogen of wood during decay.

3. Comparisons between the amount of wood disappearing during decay and the maximum amount of nitrogen available indicate that the amount of wood is 500–700 times as great as the total nitrogen available.

4. Considerations of the effects of added nitrogen indicate that the nitrogen present is utilized more effectively during decay than is that added.

5. The efficiency of certain fungi in utilizing nitrogen may explain in part their great success in destroying wood.

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STARCH HYDROLYSIS IN BEAN LEAVES AS AFFECTED BY APPLICATION OF GROWTH-REGULATING SUBSTANCES

JOHN W. MITCHELL[1] AND MURIEL R. WHITEHEAD[2]

(WITH ONE FIGURE)

Introduction

In a previous investigation (**7**) it was noted that the rate of starch hydrolysis in attached bean leaves kept in darkness was increased as a result of application of naphthaleneacetic acid. It was also observed that the sugar content of leaves kept in darkness increased appreciably for several hours following treatment, while that of control leaves steadily decreased. When leaves were first depleted of carbohydrates, then treated and illuminated by means of natural light, they accumulated carbohydrates more slowly than did untreated leaves under comparable conditions.

In view of the fact that naphthaleneacetic acid affected the rate of starch hydrolysis and the sugar content of leaves, it was decided to study these responses in more detail and to determine the relative effectiveness of several other growth-regulating substances in inducing similar responses. Seven compounds were selected as representative of those that induce various histological and physiological responses in plants (**1, 2, 3, 4, 6, 8, 9**), including: phenylacetic, beta indole-3-acetic, gamma (indole 3)-n-butryic, beta indole 3-propionic, alpha naphthaleneacetic, and beta naphthoxyacetic acids, and alpha naphthalene acetamide. Emulsions containing these substances were sprayed on attached bean leaves, and their relative effectiveness in increasing the rate of starch digestion above that of comparable control leaves, during a period of darkness, was determined by quantitative analyses. The effect of these substances on the rate of starch digestion in relatively young as compared with mature leaves was studied, and the effect of indoleacetic acid on the sugar content of relatively young leaves kept in darkness was determined.

Methods

Attached heart-shaped leaves of kidney bean plants, Calapproved variety, were used. A great number of plants were grown under greenhouse conditions in soil contained in 4-inch clay pots for a period of 2–4 weeks. Plants were carefully selected from this supply for the experiments. Relatively bright and clear weather prevailed during the experiments, so that it was not difficult to obtain leaves with an appreciable amount of reserve carbohydrate in the form of starch. In order to

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accelerate the storage of starch in the heart-shaped leaves, all portions of the plants above the second node were removed, as was done in previous experiments (7). In treating the leaves, lanolin-water emulsions containing the different growth-regulating substances were sprayed on the upper surfaces (7).

Composite samples of fifty leaves which had first been dried in a well-ventilated oven at 80° C., washed five times with ether, ground to 100 mesh, and finally redried in a vacuum oven, were used for chemical analyses.

Sugar in the dried samples was determined in duplicate as previously described (7). The combined starch and dextrin was determined by first removing sugars from a weighed amount of the powder by means of repeated extractions with 80 per cent alcohol. The starch was then gelatinized by holding the temperature of the wet sample at 75°–80° C. for 30 minutes. After cooling, 1 ml. of fresh, filtered saliva, which had been diluted with an equal volume of water, was added, together with 10 drops of toluene, and the starch digested for 30 minutes at 35°–38° C. The sample was again heated to 75°–80° C. for 30 minutes, cooled, fresh saliva added and again digested for 30 minutes. Periods of alternate heating and digesting were continued until all the starch was hydrolyzed, as shown by the iodine tests. The sample was then filtered and cleared with lead acetate. A 50-ml. aliquot of the cleared solution was placed in a 100-ml. volumetric flask, together with 3 ml. of concentrated hydrochloric acid. The acidified solution of dextrin and the products of saliva digestion were then autoclaved for a period of 20 minutes at a temperature of 112°–114° C.

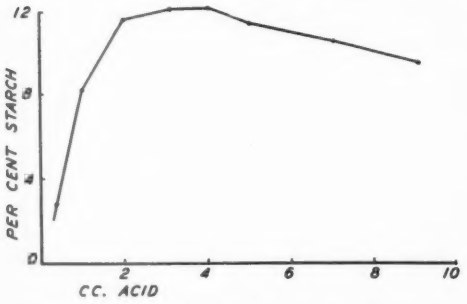

FIG. 1.—Effect of heating aliquots of a solution containing dextrin and products of saliva digestion for 20 minutes at 112°–114° F. with different amounts of hydrochloric acid. Reducing power of solutions after hydrolysis expressed in terms of combined percentages of starch and dextrin in leaf sample.

The reducing power of the solution, following hydrolysis of dextrin and the products of saliva digestion, depended largely upon the conditions under which the hydrolysis was carried out. Although the solution was heated for only 20 minutes, relatively low figures were obtained when 10 ml. of acid was used in 50 ml. of the solution to be hydrolyzed, apparently because some of the sugar was caramelized. In order to determine the optimum conditions for hydrolysis, various amounts of acid were used, and the solutions were heated for 20 minutes at a temperature of 112°–114° C. The results of this experiment show that maximum reducing power resulted when the solution containing dextrin and the products of

saliva digestion was hydrolyzed by adding 3 ml. of acid to 50 ml. of solution (fig. 1). The pH of the cleared solution with this amount of acid added was approximately 0.35.

In order to determine whether 20 minutes was sufficient time for heating, quadruplicate samples were prepared and 3 ml. of acid was added to each. Two were then heated for a 20-minute period, while the others were heated for 1 hour. No difference was noted in the reducing power of the final solutions.

These data show that maximum reducing power resulted when 3 ml. of acid was added and the solutions heated at 112°–114° C. for 20 minutes. These conditions were considered optimum for this method of hydrolysis, and were therefore used in all the starch determinations. The reducing power of the sugar solutions following acid hydrolysis was determined as previously described (**5**). As a matter of simplicity the combined starch and dextrin content of samples is reported in the tables as starch.

Results and discussion

Preliminary experiments were carried out to determine the optimum temperature at which starch hydrolysis could be stimulated in leaves as a result of application of a growth-regulating compound. One hundred forty-four young plants were selected and divided into six equal groups. Leaves of the plants in three of these lots were then sprayed with an emulsion containing 1 per cent indoleacetic acid (percentage calculated on basis of weight of lanolin used, **7**). The remaining three groups were sprayed with pure lanolin emulsion. Each group of treated plants, together with an equal number of controls, was then placed in darkness in separate rooms. The temperature of one room was controlled at 62°–64°, another at 74°–76°, and the third at 90°–92° F. By means of iodine tests the starch contents of control and treated leaves kept at the different temperatures were compared at intervals during the following 12-hour period of darkness. Neither treated nor control leaves hydrolyzed an appreciable amount of starch at the low temperature. Treated leaves at 74°–76° F. gave a negative starch test at the end of 12 hours of darkness, while control leaves kept at the same temperature showed a positive test. Approximately all the starch was hydrolyzed in both treated and control leaves kept at 90°–92° F. for 12 hours, and at no time during the experiment was there a noticeable difference between the amount of starch in treated as compared with control leaves kept at this temperature. Evidently the application of indoleacetic acid failed to stimulate the relatively slow diastatic activity in leaves maintained at a low temperature, nor was there a noticeable increase in the rapid rate of starch digestion in leaves kept at a high temperature; but its use did result in a definite increase in the diastatic activity in leaves kept at the moderate temperature of approximately 75° F. Subsequent experiments concerning the effect of this

and other growth-regulating compounds on diastatic activity were therefore carried out with a controlled temperature of 74°–78° F.

Before studying quantitatively the effectiveness of different growth-regulating chemicals on the rate of starch digestion, the optimum concentration of each substance to be used was determined. Emulsions were prepared which contained the substances to be studied in concentrations of 0.01, 0.1, 1, and 5 per cent of the weight of lanolin in the emulsion. Leaves were then treated with these emulsions, the plants placed in darkness, and the rate of starch hydrolysis determined by means of iodine tests made at intervals during a period of 12 hours. Optimum effects without apparent injury were observed when 1 per cent emulsions were used, with the exception of phenylacetic acid, in which case 0.1 per cent was most effective. As a result of these tests these optimum concentrations were used in all subsequent experiments.

In order to study the effect of various growth-regulating chemicals on starch digestion in immature leaves, a number of young plants which had accumulated a relatively small amount of reserve carbohydrates were selected and divided into nine equal groups. The plants of one lot were treated with pure lanolin and the leaves were immediately harvested, so that the starch content of representative leaves at the beginning of the experiment could be determined. Plants of another group were also treated with pure emulsion but harvested at the conclusion of the experiment as final controls. The remaining groups were treated individually with indoleacetic, naphthaleneacetic, indolepropionic, phenylacetic, indolebutyric, and naphthoxyacetic acids, and naphthalene acetamide. The plants were then kept in darkness for 12 hours, after which time the leaves were harvested and analyzed for starch.

This experiment was repeated in identically the same way except that older, more mature plants, which had accumulated a relatively large amount of reserve carbohydrate in the form of starch, were used. Diastatic activity in the relatively mature leaves sprayed with pure lanolin emulsion was not appreciably different from that of young rapidly growing leaves treated in the same way. This is evidenced by the fact that 64 per cent of the starch and dextrin was digested in the leaves of the former and 66 per cent in the leaves of the latter during a 12-hour period of darkness (table 1). However, the rate of starch digestion in the less mature leaves, which initially contained 9.7 per cent starch on the basis of solid matter, was definitely increased as the result of treatment with emulsions containing indoleacetic, naphthaleneacetic, indolepropionic, indolebutyric, and naphthoxyacetic acids. It was only slightly stimulated as a result of treatment with phenylacetic acid, while an emulsion containing naphthalene acetamide was ineffective under the conditions of this experiment.

When older leaves—which initially contained 20.4 per cent starch—were used,

application of the chemicals, with the exception of indoleacetic acid, resulted in a decrease in the rate of starch digestion during the 12-hour period of darkness immediately following treatment. The rate of starch digestion in the older leaves treated with indoleacetic acid was not appreciably different from that of control leaves.

Repeated experiments were carried out with leaves of young plants that were approximately 15 days old and which contained a relatively small amount of reserve carbohydrate in the form of starch, and also with mature leaves of plants approximately 30 days old which contained a larger amount of reserve carbohydrate. Data obtained substantiated the results of the former experiments, in that starch digestion was increased in the immature leaves as the result of treatment

TABLE 1

STARCH HYDROLYSIS IN RELATIVELY YOUNG AS COMPARED WITH MATURE BEAN LEAVES FOLLOWING APPLICATIONS OF GROWTH-REGULATING SUBSTANCES

Treatment	Percentage of initial starch content hydrolyzed during 12-hour period of darkness		Treatment	Percentage of initial starch content hydrolyzed during 12-hour period of darkness	
	Young leaves	Mature leaves		Young leaves	Mature leaves
Untreated		62	Indolepropionic acid	86	44
Pure lanolin	66	64	Naphthoxyacetic acid	87	48
Naphthalene acetamide	66	49	Naphthaleneacetic acid	88	56
Phenylacetic acid	71	42	Indolebutyric acid	90	59
Indoleacetic acid	85	66			

and was slightly retarded in the mature leaves during a period of 12–24 hours of darkness. The leaves of the more mature plants failed to show epinastic responses, although those of the younger plants became curled and bent within 1 hour following treatment with emulsions containing indoleacetic, indolepropionic, naphthaleneacetic, and indolebutyric acids.

It is evident that the application of a number of different growth-regulating compounds increased the rate of starch digestion in young immature leaves. Applied to older, more mature leaves under the same conditions, these chemicals retarded diastatic activity. It would appear from these results that the effect of these chemicals on the rate of starch digestion was controlled to some extent by factors or conditions which varied with the relative maturity of the plant.

In previously reported experiments (7) using naphthaleneacetic acid, there was associated with starch digestion a marked but temporary increase in the percentage of sugar present in leaves kept in darkness. In the present investigation ex-

periments were carried out to determine whether leaves treated with indoleacetic acid gave a similar response. A number of selected plants were treated with an emulsion containing 1 per cent indoleacetic acid and placed in darkness, together with controls which were treated with pure lanolin emulsion. Leaves were collected at intervals during the following 15-hour period and analyzed for total sugars. The percentage of sugar in leaves treated with indoleacetic acid increased to twice that of control leaves within a period of 4 hours after treatment (table 2) and remained at a relatively high level for several hours. At the end of 15 hours of darkness, the sugar content of treated leaves had decreased so that it was again approximately equal to that of control leaves, and at the same time the starch content of treated leaves decreased to less than 1 per cent of the dry weight.

TABLE 2

SUGAR CONTENT OF LEAVES SPRAYED WITH INDOLEACETIC ACID COMPARED WITH THAT OF CONTROL LEAVES

Hours after treatment	Averages of duplicate determinations of percentage sugar (dry weight)		Hours after treatment	Averages of duplicate determinations of percentage sugar (dry weight)	
	Control	Treated		Control	Treated
0*	2.6	2.6	11	3.3	4.5
4	2.4	4.9	13	1.9	3.0
7	2.9	4.3	15	1.7	1.9
9	2.6	4.5			

*Sampled at time of treatment.

These results demonstrate that the treatment of leaves with indoleacetic acid resulted in a marked increase in their sugar content, during a period of darkness, as had already been observed in leaves treated with naphthaleneacetic acid. It is not known whether this increase in sugar in the leaf resulted from carbohydrate transported from other parts of the plant, or whether it resulted from the hydrolysis of more complex carbohydrates.

Summary

1. Lanolin emulsions containing various growth-regulating chemicals were sprayed on attached bean leaves and the plants were subsequently placed in darkness, together with controls. Quantitative analysis of the starch and dextrin content of the leaves showed that application of sprays containing indoleacetic, naphthaleneacetic, indolebutyric, indolepropionic, and naphthoxyacetic acids resulted in marked increase in the rate of starch digestion. Phenylacetic acid resulted in only a slight increase, while naphthalene acetamide had no noticeable effect.

2. Treatment of relatively old and mature leaves with these various chemicals either failed to stimulate, or inhibited to some extent, the hydrolysis of starch during a 12-hour period of darkness following treatment.

3. Application of these growth-regulating chemicals did not result in a noticeable increase in the rate of starch digestion in leaves kept at 62°–64° or at 90°–92°, but was effective at 74°–76° F.

4. The sugar content of leaves kept in darkness increased appreciably for a period of time following treatment with indoleacetic acid, a response previously observed in leaves treated with naphthaleneacetic acid.

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ONTOGENY OF MEDULLARY BUNDLES IN APIUM GRAVEOLENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **519**

EDITH C. LAMBETH

(WITH TWELVE FIGURES)

Introduction

The occurrence of medullary bundles has been reported (**5**) for many species of the Umbelliferae, but there seems to be no uniformity in the number, arrangement, or differentiation of the bundles. NESTEL (**4**) mentioned the presence of small, isolated conducting bundles in the pith, proximal to the common vascular ring; he also observed one such bundle in the cortex. According to his description, they are inclosed in a lignified sheath of bast (libriform) wood parenchyma. The phloem is well developed while the xylem is limited, consisting of small vessels. This and other general references state that medullary bundles are found in many of the Umbelliferae, but the writer has found no report as to the origin and development of these anomalous structures. The present study was undertaken to determine the ontogeny of the medullary bundles in the flower stalk.

Material and methods

Two varieties of celery (*Apium graveolens* L.) were used, the White Plume and the Golden Self Blanching. The plants were grown in the greenhouse and were harvested at different stages during flowering. Navashin's solution and formalin-alcohol-acetic were employed as fixing agents. Best results were obtained with material treated by a tertiary butyl alcohol series, rather than by xylol or chloroform. All sections were cut on a rotary microtome at 10 μ. Star razor blades, clamped in a Spencer razor-blade holder, worked satisfactorily for sectioning. A modification of the triple stain was used.

Observations

The medullary bundles tend to be developed opposite the protoxylem points of the common vascular bundles (**1**). The earliest stage in their ontogeny is indicated by meristematic activity in the parenchymatous cells near the periphery of the pith (fig. 3). From the cells thus derived, an oil duct is formed as a schizogenous, intercellular space. Those cells, generally three (fig. 2), bordering directly on the duct are thin walled and epithelial in nature (**2**). Anticlinal divisions may

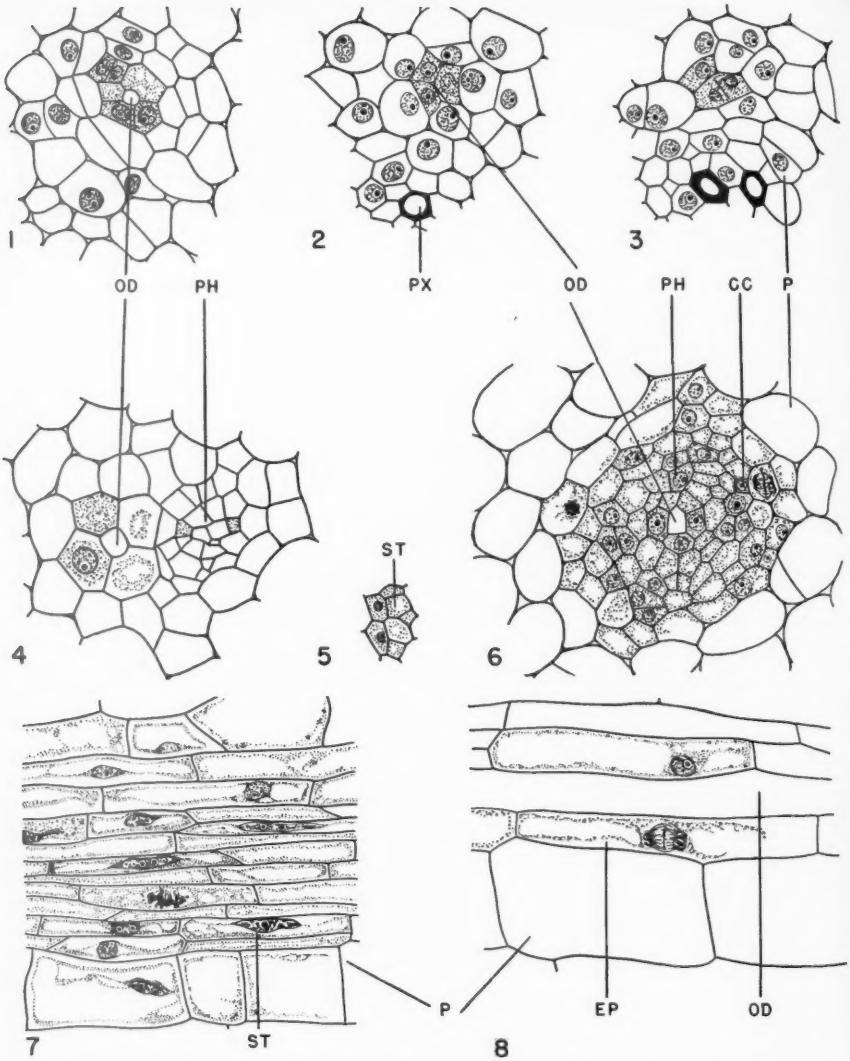
ultimately result in the formation of four to six epithelial cells, as shown in transverse view (fig. 1). Elongation of the duct and surrounding cells takes place by means of transverse divisions of the component cells (fig. 8).

Coincident with the differentiation of the epithelial cells, divisions also occur in adjacent parenchymatous cells (fig. 6). Characteristic of meristematic regions, these cells show prominent nuclei and darkly stained cytoplasm. Anticlinal, periclinal, and transverse divisions occur in rapid succession, and the derivatives differentiate as phloem. Development of the phloem in the medullary bundles parallels that of the phloem in the vascular bundles of the petiole (2). A parenchymatous cell undergoes several divisions, the second before the last giving rise to a phloem parenchyma cell and a sieve tube-companion mother cell. The sieve tube-companion mother cell divides, and the larger of the two resulting cells is the sieve tube (fig. 5). The smaller cell may give rise to one or more companion cells by transverse division. Figure 7 shows a phloem element dividing tangentially. Essentially the same stage of development is shown in transverse section in figure 6.

Although earlier divisions are not uniform on all sides of the epithelial cells (fig. 4), meristematic activity is established so that phloem finally surrounds the entire oil duct. Thus one oil duct generally becomes central in position. Frequently two ducts are differentiated so close together that phloem may become differentiated in the parenchyma that originally separated them, thus producing a bundle having two oil ducts. As many as five have been observed within a single bundle. Exceptionally, a duct was found situated adjacent to a row of cambium cells which had already differentiated xylem on the periphery.

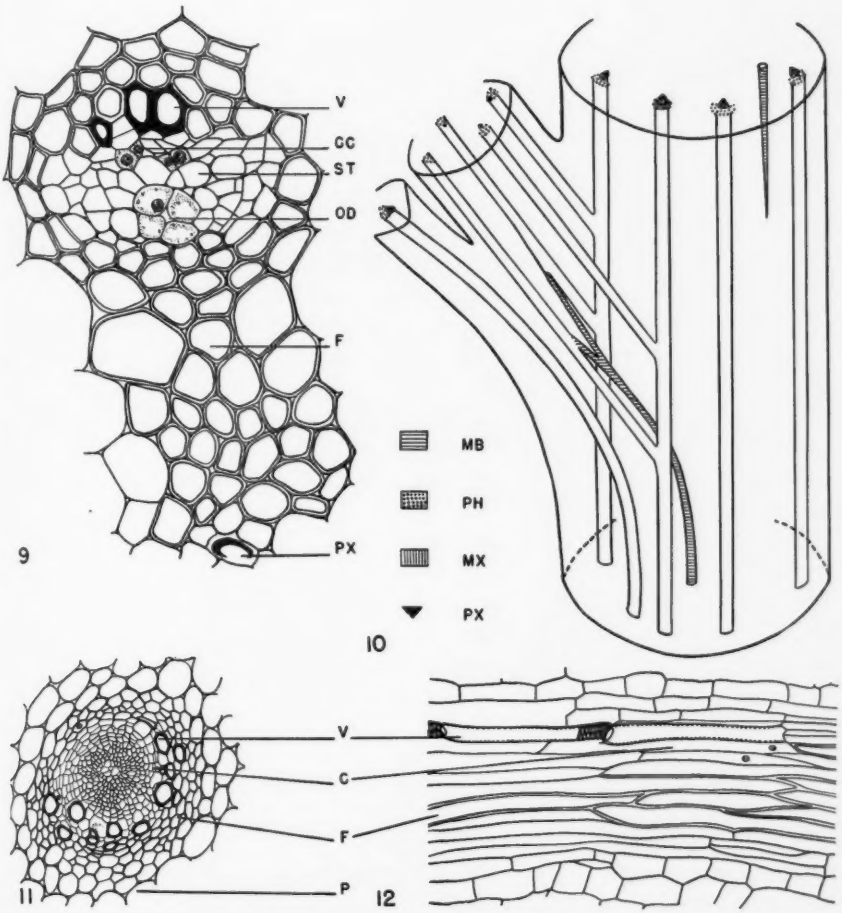
As the stem matures the active parenchymatous cells surrounding the phloem cylinder differentiate as a ring of cambium cells, two to four cells in thickness. From the derivatives of the cambium, secondary xylem and additional phloem elements are differentiated. The secondary xylem consists primarily of fibers, but in most bundles vessels occur scattered irregularly among the fibers, or arranged in discontinuous rows (fig. 11). Tapering ends and thick walls, with very small but abundant pits, characterize the fibers. Secondary wall thickening of the xylem elements is primarily of the scalariform type. Some reticulate thickenings were observed. In figure 12 the vessel is cut at such an angle as to show the disintegrated end wall (3).

The bundles in very mature stems are characterized by a greater development of fibers. This condition is brought about by the maturation of cambial derivatives into fibers which border directly on the phloem elements. In some cases a single row of cambium cells may remain. Frequently the parenchyma cells separating a medullary bundle and a common bundle may differentiate as fibers until direct contact is established between the two bundles (fig. 9). Many bundles were ob-



FIGS. 1–8.*—Figs. 1–6, transections showing stages in development of medullary bundle: 1, divisions in adjacent parenchyma cells; 2, oil duct surrounded by three epithelial cells; 3, parenchymatous cells opposite protoxylem point, dividing (figs. 1–3 from same bundle at different levels); 4, phloem development prominent on one side; 5, phloem detail; 6, phloem elements surrounding oil duct and divisions apparent on periphery. Fig. 7, longisection through a young bundle showing phloem element dividing tangentially. Fig. 8, same through oil duct showing epithelial cell dividing.

* Abbreviations for figures: *c*, cambium; *cc*, companion cell; *ep*, epithelial cell; *f*, fiber; *mb*, medullary bundle; *mx*, metaxylem; *od*, oil duct; *st*, sieve tube; *v*, vessel; *px*, protoxylem; *p*, parenchyma.



FIGS. 9–12.—Fig. 9, transection through mature medullary bundle fused with protoxylem point of common vascular bundle by means of xylem fibers differentiated from parenchyma cells that separated them. Fig. 10, diagram of sector through node near base of umbel, showing two medullary bundles in their relation to the common vascular system. Fig. 11, transection through mature medullary bundle. Fig. 12, longisection through mature medullary bundle.

served to arise *de novo* and terminate in the pith (fig. 10). The phloem at the center of the mature bundles often appears crushed, and in some cases the epithelial cells show signs of deterioration.

The medullary bundles are cauline in nature. Through the internodes they follow a straight course, but at the nodes they may be distributed in any one or more of several ways. Those bundles situated on the side opposite a lateral branch may follow a straight course through several internodes. A bundle situated on the side of the stem giving rise to a lateral branch, however, may show anastomosing with another medullary bundle, branching, or differentiation so close to the phloem of the common bundle that the elements composing it are in contact with the phloem of the common bundles of the lateral branch. This last possibility is illustrated in figure 10, which shows that the phloem of the medullary bundle differentiates in contact with the phloem of the vascular bundles situated on the abaxial side of the lateral branch.

Contrary to the more common derivation of medullary bundles in dicotyledons (6), the bundles in celery show no direct relation to the leaf traces. Generally the term "medullary bundle" is used to describe intraxylary or internal phloem. Various theories as to the derivation and phylogenetic significance of these bundles have been advanced. According to WORSDELL (7), "the morphological origin of this internal phloem bundle is from an amphivasal bundle, for this latter is the typical and primitive condition of medullary bundles wherever they may occur." As quoted by WORSDELL, LAMOUNETTE "concludes (not only for Cucurbitaceae, but for other orders investigated) that internal phloem is an abnormal formation due to the activity of certain cells of the central conjunctive parenchyma, or is the ulterior evolution of the axis." This mode of development of the medullary bundle is essentially in agreement with the findings of the present investigation, but the phylogenetic significance of such bundles remains in question.

Summary

1. A parenchymatous cell or several cells opposite the protoxylem point of any vascular bundle become meristematic. From the cells thus derived, an oil duct is formed schizogenously.

2. Parenchymatous cells surrounding the epithelial cells of the oil duct become meristematic. Anticlinal, periclinal, and transverse divisions occur in rapid succession, and the derivatives differentiate as phloem.

3. Although earlier divisions are not uniform on all sides of the epithelial cells, such meristematic activity is usually established that phloem finally surrounds the entire oil duct. Later a cambium is differentiated from the active parenchymatous cells surrounding the phloem. From the derivatives of the cambium secondary xylem and additional phloem elements are differentiated.

4. The medullary bundles are cauline in nature. Initiation of these bundles may be *de novo* at any level of the stem and terminate at any level. Frequently, at the nodes, they differentiate so close to the phloem of the common bundle that the elements composing them are in contact with the phloem of the common bundles of a lateral branch.

5. There is no direct connection of the medullary bundles with the leaf traces.

Grateful acknowledgment is made to Dr. H. E. HAYWARD for suggesting this problem, and to Dr. E. J. KRAUS for helpful suggestions during the progress of the investigation.

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ABSORPTION SPECTRA OF THE CAROTENOIDS IN THE RED AND BROWN FORMS OF A PHOTOSYNTHETIC BACTERIUM

C. S. FRENCH

(WITH ONE FIGURE)

Introduction

Streptococcus varians, a species of purple bacteria capable of photosynthetic CO_2 reduction, if hydrogen is available, will when grown in the absence of O_2 form a deep brown culture in yeast extract medium. If air or O_2 is admitted during or after the growth period the culture becomes bright red in a few hours. The addition of oxidizing or reducing substances to a suspension of the cells or to the water extracts described later does not bring about this color change rapidly, nor has the red form been changed back to the brown. Water extracts of the cell juice from brown cells remain brown for days when in contact with air. It therefore seems likely that the brown to red change is an enzymatic process taking place only in the intact bacteria and possibly having some relation to cell respiration. These bacteria contain two types of pigments: green methyl alcohol-soluble bacteriochlorophyll (**1, 9**) and reddish acetone and fat solvent-soluble carotenoids (**6, 10**). Only the former is used as the light absorber for photosynthesis (**3**), while the function (if any) of the latter is, like the use of the carotenoids in green plants, still unknown. A knowledge of these spectral changes in photosynthetic bacteria may have a bearing on the general problem of carotenoid function. The absorption spectra reported here afford a basis for a chemical investigation of this phenomenon and in themselves allow some speculation as to its nature. Furthermore, since quantitative photochemical measurements of the assimilation of carbon dioxide and hydrogen by near infra-red radiation have been made with this species (**2**), it is of interest to know the absorption spectra of the different colored forms.

From these measurements it appears probable that the change from brown to red when O_2 is allowed to come in contact with anaerobically grown bacteria is due either to the formation of an extra pigment with an absorption band having a maximum at 550 mμ or to the modification of an existing carotenoid, so that it has an extra band at that place.

Experimentation

In order to make precise spectral absorption measurements of the bacterial pigments in the cells, it is advantageous to have a clear solution of the pigments,

so that scattering of light by the cell walls can be avoided. Extraction by organic solvents changes the position of the absorption bands of leaf and photosynthetic bacteria pigments. This group of bacteria, however, like some green plants, contain their colored components attached to a water-soluble protein (**7, 8, 9**). This protein pigment complex, photosynthin, may be obtained in a clear and fairly stable solution by breaking open the cell walls of a suspension of bacteria (**4, 5**).

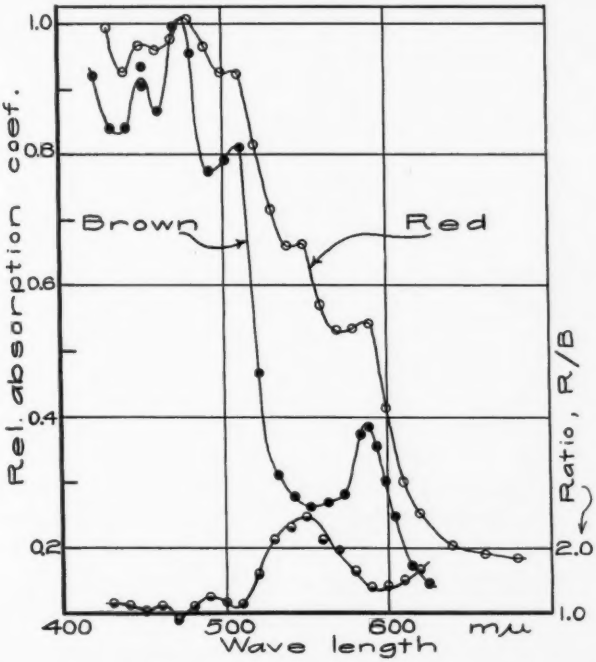

FIG. 1.—Absorption spectra of red (upper curve) and brown (lower curve) forms of *Streptococcus varians*. Ratio of curves shown at bottom of figure.

A thick suspension of *Streptococcus* (*Rhodopseudomonas* or *Phaeomonas*) *varians* (strain Original[1]) was put in a glass hypodermic syringe cylinder whose tip was sealed with de Khotinsky cement. The plunger was then inserted. The cylinder was held in the chuck of a lathe and rotated slowly while the plunger was gently forced in and kept from revolving by being fixed to the tail stock. The liquid suspension was squeezed out through the space between the well-fitting glass surfaces. A small percentage of the bacteria emerging were broken open and their

[1] This strain does not assimilate H_2, at least under conditions favorable for that reaction in the strain designated as C11 by VAN NIEL, although it appears spectroscopically identical.

cell juice liberated into the surrounding liquid. This liquid was then centrifuged, the supernatant fluid diluted with water, and the absorption curve measured in the visible region with a Koenig-Martens spectrophotometer. Curves were made both for extracts of the brown bacteria grown as usual without air and the red bacteria from the same strain grown with air bubbling through the culture. As the percentage of cells broken by this treatment was not controllable, the curves are not comparable as to absolute height but are brought together at their maxima. Figure 1 shows these curves.

Results

The bands at 590 mμ are due to bacteriochlorophyll and the others to the carotenoid pigments, as has been established by extraction experiments. The red bacteria show an extra absorption band at 550 mμ and less sharp bands of the carotenoid pigment. The apparent raising and blurring of the 590 mμ bacteriochlorophyll band is evidently due to the increased absorption in this region by part of the 550 mμ band. The bottom curve with half-filled circles is the ratio of the upper two curves and may be interpreted as the relative absorption curve of an extra pigment produced by the action of oxygen on the brown bacteria. It is possible, however, that some of the carotenoid pigment present in brown bacteria is altered by the action of O_2, making it absorb at longer wave lengths so that the effect might be attributed equally well to a modification of a pigment existing also in the brown type. This interpretation is favored by the blurring of the three carotenoid bands. So far no attempts have been made to separate this hypothetical extra pigment and study its chemical nature. Its absorption spectrum would suggest that it was not a carotenoid.

Summary

1. Bacteria may be ground with a hypodermic syringe to liberate the water-soluble constituents.

2. Both aerobically grown (red) and anaerobically grown (brown) extracts of *Streptococcus varians* thus prepared have been examined spectroscopically.

3. An extra band at 550 mμ is found in the red form.

This work was done at the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts.

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DESMIDS OF MIRROR LAKE, UTAH

EDNA SNOW

Mirror Lake is one of many glacial lakes located in the Uinta Mountains of Utah. The water of the lake is acid, having a pH of about 5.1, according to TANNER (6). The collections of algae from this lake were made during August and

TABLE 1

Species found	No. of collections	Species found	No. of collections
Netrium digitus var. constrictum W. West	1	Cosmarium margaritiferum Menegh.	1
Penium spirostriolarum Barker	1	Cosmarium subspeciosum var. validium Nordst.	1
Gonatozygon brebissonii De Bary	1	Cosmarium subturgidum fa. minor Schmidle	1
Closterium abruptum W. West	2	Cosmarium costatum Nordst. Fa.	1
Closterium costatum Corda	2	Cosmarium subcucumis Schmidle	1
Closterium moniliferum (Bory) Ehr.	2	Cosmarium hammeri var. protuberans W. & W.	1
Closterium lanceolatum Kutz. var.	1	Cosmarium subcrenatum var. isthmochondrum	1
Closterium lineatum Ehr.	1		
Closterium parvulum Naeg.	1	Staurastrum ophiura Lund.	1
		Staurastrum grande Bulnh.	1
Pleurotaenium ehrenbergii (Breb.) De Bary	1	Staurastrum natator var. crassum W. & W.	1
Pleurotaenium coronatum var. nodulosum (Breb.) West	1	Staurastrum vestitum Ralfs	1
Pleurotaenium trabecula (Ehr.) Naeg.	1	Staurastrum polytrichum Perty	1
Pleurotaenium truncatum (Breb.) Naeg.	1	Staurastrum punctulatum Breb.	1
		Euastrum verrucosum var. rhomboideum Lund.	4
Cosmarium crenatum Ralfs	1	Euastrum gemmatum Breb.	2
Cosmarium bioculatum Breb.	1	Euastrum elegans (Breb.) Kutz.	5
Cosmarium meneghinii Breb.	1	Euastrum bidentatum Naeg.	2
Cosmarium subcrenatum Fa.	5	Euastrum ansatum Ehr. var.	2
Cosmarium turpinii Breb.	1	Euastrum didelta (Turp.) Ralfs	2
Cosmarium turpinii var. exmium W. & W.	2		
Cosmarium angulosum var. concinnum W. & W.	1	Sphaerozosma granulatum Roy et Biss.	1
Cosmarium margaritatum (Lund.) Roy et Biss	2	Hyalotheca mucosa (Mert.) Ehr.	1
Cosmarium punctulatum var.	1	Micrasterias americana (Ehr.) Ralfs	1

September of 1937 and 1938. Algal forms were less abundant here than in many of the more shallow lakes, although they were plentiful near the north end, where a small stream of water enters. Desmids were the most common form of algae

collected. They were found associated with other green algae on the muddy bottom at depths of 4–12 feet.

Very little work has been done on the algae of Utah. HARRISON (1), NORRINGTON (2), TANNER (5), and SNOW (3) reported algae from various regions in the state. From the available lists, only six of the species of algae listed in table 1 have been previously reported from Utah. NORRINGTON reported *Pleurotaenium trabecula* (Ehr.) Naeg.; SNOW (4) reported *Closterium moniliferum* (Bory) Ehr., *Pleurothaenium threnbergii* (Breb.) De Bary., *Euastrum elegans* (Breb.) Kutz., and *Micrasterias americana* (Ehr.) Ralfs; and TANNER (5) reported *Euastrum verrucosum* Ehr. but not the variety *rhomboideum* Lund. included here.

Table 1 gives the species collected. The number following each form indicates the number of collections in which each species was found, roughly indicating the relative abundance of the species. Eleven genera and forty-four species were found. PRESCOTT reports *Staurastrum natator* var. *crassum* W. & W. to be a new form. Identification of all species was made by G. W. PRESCOTT of Albion College, Michigan. The writer is greatly indebted to him and also to Dr. B. F. HARRISON, of Brigham Young University, for their help and encouragement.

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CURRENT LITERATURE

The New Systematics. Edited by JULIAN HUXLEY. Oxford: Clarendon Press, 1940. Pp. viii+583. Figs. 57. $6.00.

The relation of various biological sciences to taxonomy is discussed by various authors, mostly Englishmen. Chapters dealing mainly with botanical material include: Experimental and synthetic plant taxonomy, by W. B. TURRILL; Taxonomic species and genetic systems, by C. D. DARLINGTON; Ecological aspects of plant taxonomy, by E. J. SALISBURY; Taxonomic problems in fungi, by J. RAMSBOTTOM; Taxonomic botany, with special reference to the angiosperms, by T. A. SPRAGUE; Natural hybridization in relation to taxonomy, by H. H. ALLAN; Origin and behaviour of cultivated plants, by M. B. CRANE; and The new systematics of cultivated plants, by N. I. VAVILOV. J. S. HUXLEY brings the various fields, both zoological and botanical, together in a well-balanced introductory chapter.

The major factoral complexes involved in speciation include hereditary variation, isolation, and selection of natural populations. The action of these factors among cultivated plants shows the reciprocal principles involved in artificial and natural evolution. Both plants and animals are under fundamentally similar directive forces, the major difference being the gross chromosomal differences often associated with speciation in asexual, parthenogenetic, or self-fertilized organisms.

There is considerable difference among the species definitions by the various authors. When the evidence from all the correlated fields is summarized, however, it would seem that the species may be defined as that evolutionary stage at which a genetically distinctive, reproductively isolated, natural population becomes established. From both a theoretical and practical standpoint, such a definition conforms to the general practice of biologists working in various fields and fits the large number of species sufficiently well studied. Difficulties in the application of such a concept appear in the few species which show gradual hereditary change in complete paleontological series, and among the asexual populations where reproductive isolation is established with the origin of the individual and slight hereditary change occurs gradually, as in strains of bacteria.

The book is a landmark in progress toward collaboration of scientists from various divisions of biology. Although divergent views are often found among specialists, any attempt to formulate fundamental principles tends to bring these differences of opinion into the open, where they may be subjected to critical evaluation based upon evidence from many directions.—A. E. EMERSON.

A Manual of Aquatic Plants. By NORMAN C. FASSETT. New York: McGraw-Hill Co., 1940. Pp. vii+382. Illustrated. $4.00.

Taxonomists have been very conservative in making the facts of their science readily available for the use of laymen or scientists untrained in systematic botany. A departure from this situation has become imperative in view of the increasing need for quick and accurate plant identification in the field by workers in the branches of wildlife, grazing, forestry, and soil conservation. Aquatic biologists, including experienced botanists, have reason to be grateful for the

present volume, which leads the field in this direction. A competent taxonomist has devoted much time and effort to compiling simple but accurate artificial keys, closely supplemented by original drawings illustrating all technical points, which facilitate quick identification of most macroscopic plants, whether sterile or flowering, normally germinating and growing with at least their bases in water. The territory covered ranges from Missouri and Minnesota to Virginia and the Gulf of St. Lawrence. The flora of bogs, small woodland brooks, waterfalls, and tidal, salt, and brackish waters is not included.

Part I, 33 pages, is a general key, based entirely on vegetative characters, which leads to the family, genus, or species name, and reference to the descriptive treatment in part II, 306 pages. This in turn is primarily a key to genera and species under each family, but in some cases necessarily requires the use of flower or fruit characters. Brief notes or descriptions are given for some families and genera, but most of the descriptive treatment of them, as well as of species, is given in the keys. A short statement of habitat and range follows most species' names. In spite of this brief textual treatment, there is rarely need for more adequate confirming description than is supplied by reference to the excellent and profuse illustrations, drawn with emphasis on the key characters. Families follow the ENGLER and PRANTL sequence, but genera may be grouped for the sake of convenience, as in the grasses, where they are placed in the order of their importance to the aquatic biologist. His interests are also considered in the appendix, which is a compilation, from cited papers, of the uses of aquatic plants by birds and mammals and of the relations of plants to fish.—C. E. OLMSTED.

Die Methoden der Fermentforschung. Leipzig: Georg Thieme, 1940.

This ambitious undertaking, which attempts to cover the field of enzyme research, is edited by EUGENE BAUMANN of Tübingen and KARL MYRBÄK of Stockholm. The prospectus indicates about 3500 pages, 800 figures, and reference to about 6000 titles. The work is deployed in three main sections, following the introduction: a general section which includes the substrates and enzymes; a special section which covers the hydrolases, desmolases, assimilation, antienzymes, and enzyme models; and a third section devoted to industrial and clinical problems involving enzyme research.

Only one part has been distributed. This part carries the first 172 pages and contains the following contributions: Simple synthetic substrates of the lipases, by EUGENE BAUMANN; The naturally occurring glycerides, by KARL HUGO BAUER; Acetylcholine, by ROBERT AMMON; Gallotannin and the simple substrates of tannase, by OTTO TH. SCHMIDT; Chlorophyll, by HANS FISCHER; Simple esters of phosphoric acid, by EBERHARD HACKENTHAL and MARIA KOBEL; Inositephosphoric acid, by THEODOR POSTERNAK; Phosphatides and their hydrolytic products, by SERAFINO BELFANTI, ALBERTO ERCOLI, and MARIA FRANNCIOLI; Simple esters of sulphuric acid, by HACKENTHAL and KOBEL; Biologically important carbohydrates and glycosides, by E. L. HIRST and L. PEAT; Extraction of natural heterosides, by J. RABATÉ; Synthetic glucosides, by HORST ELSNER; and part of a paper on thioglucosides, by FRITZ WREDE. The standing and ability of these authors guarantee the quality of the compilations and discussions. If conditions in Europe permit the completion of this cooperative venture, it should be an extremely valuable aid and stimulus to enzyme research throughout the world.

Subscribers are reminded that the publisher considers that an order placed for the first section implies that all will be accepted and paid for. The various sections cannot be sold separately. An impressive beginning has been made, and it is hoped that the entire work may become available.—C. A. SHULL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. First Supplement (pp. 24) *and Botanical Index* (pp. 82). 120 Broadway, New York: Chilean Nitrate Educational Bureau, Inc. 1940.

During recent years the Chilean Nitrate Educational Bureau has rendered an important service to nutritional research in the publication of three editions of this volume. The third edition, issued in 1939, listed more than 4600 references.

In a rapidly growing field it is out of the question to keep the bibliography up to date by reprinting the entire work at frequent intervals. To offset this difficulty, the Bureau has instituted the publication of supplements to the third edition. The first of these supplements contains 82 pages, including the indices. The references are listed by elements, in alphabetic order, a most convenient arrangement.

In addition to this service, a botanical index of the third edition has been prepared, which lists the plants in alphabetic order, together with all the nutrient elements which have been tested with each plant, listed alphabetically under each plant name, usually the common name. This renders the whole work more useful, and the volume becomes an indispensable tool for rapid information about any plant or any element. This feature is also carried over into the supplements. The first supplement has three sections to its index: first, by elements; second, a botanical index; and third, an author index.

The Chilean Nitrate Educational Bureau deserves the thanks of all workers in plant and animal nutrition for this valuable contribution to their research.—C. A. SHULL.

Leaves and Stems from Fossil Forests. By R. E. JANSSEN. Springfield: Illinois State Museum, Popular Science Series 1:1–190, 1939. Figs. 165. $1.50.

Some Fossil Plant Types of Illinois. By R. E. JANSSEN. Springfield: Illinois State Museum, Scientific Papers 1:1–125, 1940. Pls. 28. $1.25.

These publications describe, the first in nontechnical but accurate terms and the second more scientifically, some of the more common plant fossils from Illinois. So much advance has been made in our knowledge of fossil plants that it has seemed necessary to restudy the LESQUEREUX types in the Worthen collection of the Illinois State Museum, and this task has been undertaken by JANSSEN. Some fifty-nine species have been examined and only eighteen specific names remain as given by LESQUEREUX; two species have been specifically renamed and thirteen have been found valueless as specific types.

The second publication includes a description of ten new species and varieties of fossil plants recently discovered in the Mazon Creek region of Illinois.—G. D. FULLER.

Elements of Botanical Microtechnique. By JOHN E. SASS. New York: McGraw-Hill Co., 1940. Pp. ix+222. $2.50.

This elementary manual is designed for the beginning student in the field of botanical microtechnique. Part I, which covers general principles and methods, takes up the common procedures in the selection and collection of suitable materials, killing and fixation for securing structural details, dehydration, infiltration with paraffin and celloidin, sectioning, staining of sections and whole mounts for permanent microscopic preparations. Part II deals with specific methods and gives more detailed directions for the various plant phyla. A special chapter is devoted to the construction, use, and care of the microscope, and another to photomicrography. —J. M. BEAL.

Statistical Methods. By GEORGE W. SNEDECOR. Ames, Iowa: Collegiate Press, 1940. Pp. xiii+422. Illustrated. $3.75.

The third edition of this excellent work has been improved by correction of errors appearing in the second edition, by brief additions at the ends of chapters 6 and 16 (on linear regression and binomial and Poisson distributions), and by the addition of chapter 17, on design and analysis of samplings. This last chapter is a very practical one, taking up the problems of sampling from various types of populations, size of samples, etc. Actual examples are used to illustrate the methods.

This volume is the best current source of information for those who need to examine results for their validity.—C. A. SHULL.